NEUTRON EFFECTS
ON
ANIMALS

NEUTRON EFFECTS
ON
ANIMALS

By
THE STAFF OF THE
BIOCHEMICAL RESEARCH FOUNDATION
DR. ELLICE McDONALD, Director
NEWARK, DELAWARE

BALTIMORE
THE WILLIAMS & WILKINS COMPANY
1947

Composed and Printed at the
WAVERLY PRESS, INC.
for
THE WILLIAMS & WILKINS COMPANY
Baltimore, Md., U. S. A.

FOREWORD

These reports* from various members of our staff and the effort as a whole are of the nature of a research exploration to get a general survey of the problem and to choose the most promising aspects for future exploration. These reports are by no means considered to be anything but a step to further research and are given here with the hope that they will show various aspects of attack and that they will enlarge the perspective of those who wish to join in the study.

To put this volume through the press with a number of authors on various papers of scientific endeavor, with many different kinds of illustrations has required much scrutiny and discussion. Its completion is due to the consistent efforts of our librarian, Mrs. Anne Himmelberger Longenbach, and her two efficient assistants, Misses Mary Pearce and Mary Virginia Gardner. When it came to precision and constructive criticism, their "spear knew no brother". I take this opportunity of publicly tendering my grateful acknowledgment.

ELLICE MCDONALD.

Biochemical Research Foundation,
Newark, Delaware.

* Since writing this book, we have advanced considerably in our research to explain the *mechanism of radiation* on mammalian cells. It is my regret that these findings have come too late for incorporation in this book and must wait for a later publication.

Ellice McDonald

CONTENTS

Chapter 1

RADIATION EFFECTS ON CELLS

By ELLICE McDONALD, M.D., *Director*

In these days of the atomic bomb and the possibility of atomic energy being applied to industrial organization, the study of its forces seems very pertinent. When atomic energy is applied industrially, one of the chief cares will be the protection of workers against its forces and of these the chief energy will be the neutron.

It is for this reason that we have adapted our research to this study. The present research is only a beginning, to mark the way for the future. Our concern is not only to prevent the impact of radiation by screening with walls and water to keep the neutrons from reaching the operator, but also to determine the *mechanism* of its action: for only with this knowledge can remedial and curative measures be applied after the patient has absorbed the neutrons.

Radiation effects have interested the Biochemical Research Foundation for 15 years. At first it was study in regard to X-rays and ultraviolet light effects, but for the past eight years it has been the study of forces and material produced by the cyclotron. Of all the cyclotrons, that of the Biochemical Research Foundation is the only one specially built for biochemical and biological study.

The Manhattan District requested us to undertake a study of neutron effects upon the white blood cell counts of animals in order to correlate it with a companion study of X-ray effects undertaken by them at another place. While this upset the scheme of our research plans, we felt it was our duty to accede, but did so with the proviso that those not occupied in their project should have the opportunity of continuing research without limitation, insofar as it did not interfere with the program requested by the Manhattan District. This program imposed certain restrictions upon our work, the chief being, that it required the cyclotron to produce neutrons daily at a given rate, and we, of necessity, were compelled to adjust our work to that limitation.

Our cyclotron ran without missing a day for more than a year, a record for continuous performance, we believe; anyone with any experience in running a cyclotron with its aberrations and temperamentality will know what effort and foresight that required.

So our research on neutrons was geared to another plan and we adapted our research to the other work we were doing for the Manhattan project.

For this and other reasons, the report of our work is that of preliminary studies. However, we believe that it is better to publish what we have done, whether positive or negative, so that others may gain perspective as to the general problem of neutron effects.

Although radiation is grouped under one term radiations are varied in character; visible light, ultraviolet, X-rays, γ-rays, neutrons, etc., may all be emanations or radiations, but that they have the same mechanism for producing their results is a conclusion so far-fetched and erroneous as to be reached only because of the mystery of the subject. Neutron bombardment and X-radiation may produce somewhat similar results as shown in white blood cell counts and dead cells in pathologic study, but it by no means follows that they achieve these results by a similar mechanism. The cause in both cases may be radiation, and the end results—destruction of the cells—may be microscopically similar in the dead tissue, but the intermediate mechanism by which these results are attained may be quite different. It may be that neutron and X-ray effects do have a similar mechanism in producing their destructive results, but there is at present no proof that this is so.

Indeed, we have an example which shows certain lethal radiations have a mechanism which is probably quite different from X-rays. Certain short wave lengths of ultraviolet light are extremely lethal to cells when brought into their proximity and act in an apparently different mechanism from X-rays.

In 1934, we set up an all-quartz microscope and made a monochromator attachment so that photographs could be taken of the effects of various wave lengths of ultraviolet light measured in Ångstrom units. We also made moving pictures of the killing of motile cells such as euglena and paramecia in order to study the mechanism of their destruction. Cessation of their movement showed that they were killed.

We found that certain wave lengths of short ultraviolet light were more destructive than others and this because the lethal wave lengths were absorbed by the nucleus of the cell. The absorption of the quanta may be seen by the "blackening" of the nucleus in the pictures of the euglena in Fig. 1. The three horizontal pictures are different microscopic levels of the same section. It will be seen that, until the neighborhood of wave length 2804 Å, there was no absorption of the quanta of ultraviolet light and no killing resulted. When the greatest blackening or absorption of quanta by the nucleus occurred at the line 2536 Å, the cells were killed within one or two seconds. Yet the euglena treated by X-rays required 20,000 r for 24 hours in order to produce the same lethal results. Lower than 2536 Å the absorption was not so great (1).

The line 2536 Å of ultraviolet light is the *most lethal wave length yet discovered*. It is hundreds of times more lethal to cells than high voltage X-rays. Its disadvantage is the lack of penetration through tissues.

The partial mechanism of this wave length is the absorption of the quanta by the nucleic acid of the nucleus of the cell since nucleic acid has an ultraviolet spectrographic peak, an extinction coefficient, in the neighborhood of

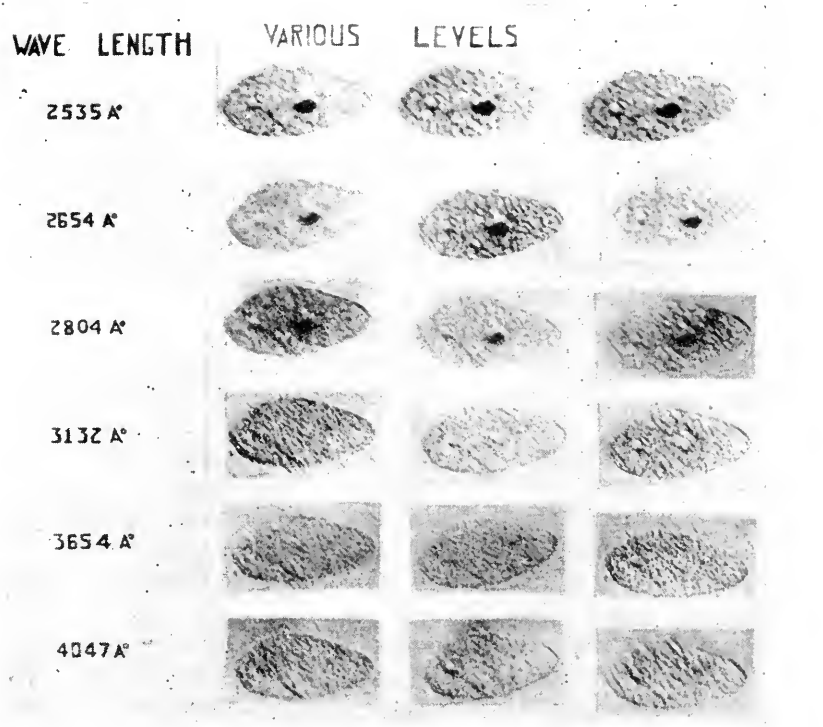


FIG. 1. Euglena killed by ultraviolet light—2535 Å

2536 Å: it is a resonance effect and a similarity of absorption coefficient. These shorter ultraviolet wave lengths apparently break down certain of the molecular bonds, particularly the polypeptide linkages which are so frequent in the amino acids.

That the same course is followed in X-ray effects seems very doubtful for there is little or no "blackening" of the cell nucleus by X-rays. It may be possible that the killing effect of X-rays is due to secondary radiation with production of wave lengths at a lower wave length, a Compton-Einstein effect, in the neighborhood of 2536 Å, but there is little or no evidence as to this. When the enormously greater energy delivered by X-rays is

considered, the comparatively slighter lethal effect is surprising so it must be concluded that only part of its energy is effective and some explanation of this fact is required.

Similar experiments have been done by us upon nucleated red blood cells of fowls which showed that the maximum absorption of the monochromatic light was at 2654 Å (2) a small deviation from the previous shorter wave length, 2536 Å.

As far as X-rays and neutrons go, there is little correlation between their mechanism upon the cells and that of the shorter ultraviolet rays. If the theory of secondary radiation at a wave length comparable to the killing wave length of ultraviolet is disproved, some other explanation must be sought.

The vital system is not as easily explained as simpler chemical reactions, but life is after all a chemical phenomenon and must be considered upon that basis. The large number of catalysts, such as enzymes, hormones and vitamins, complicate the explanation. These catalysts are specific and effective in enormously minute amounts, so that their effect is often overlooked in a general view of the vital system. No real conclusion as to effects of forces or substances can be really clear unless there is some elucidation of the mechanism involved rather than an explanation based solely on cause and end-result without consideration of the mechanism.

All chemical exchanges exist through the giving or taking or sharing of electrons, so that this must be considered as fundamental. There are three types of chemical systems. First, there are true solutions, that is strong acid and strong base, where the reactions are complete in one direction and reversible only to a perceptible degree, of the type $H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$, but this obviously cannot be the one we are concerned with in systems within the mammalian body.

Type two, another explanation of vital reactions, and it was in its time quite plausibly advanced by Arrhenius, is that of weak acid and weak base in which the equilibrium is reached while there are still present appreciable amounts of free acid and free base as well as neutral salt, the quantities depending, according to the law of mass action, upon the relative amounts in which the two reagents are present in the mixture. Attractive as this explanation is of the chemical action, particularly of immunological reactions, it has not been found acceptable, although the explanations of Pauling and others more recently suggest that character.

Colloid systems, type three, are treacherous, for the more they are dispersed, the more closely they follow the chemical form of weak acid and weak base and approach combination in stoichiometric proportion. The vital system is not only a colloid system but it is a series of colloid systems within colloid systems. It exists in a state of balance or equilibrium (not

equilibrium in the chemical sense), and this balance is necessary to the continuance of function. If it is out of balance, the system will cease to function.

The cell is the unit of life, but the cell is in an environment and the environment is part of the colloidal cellular system. The cell and its environment are one. One of the difficulties of biochemical research is that it is impossible, except perhaps in the blood, to remove a cell from its environment for study. A fish removed from its watery environment is no longer a fish that leaps and plunges but a dead thing; so also is a cell removed from its environment.

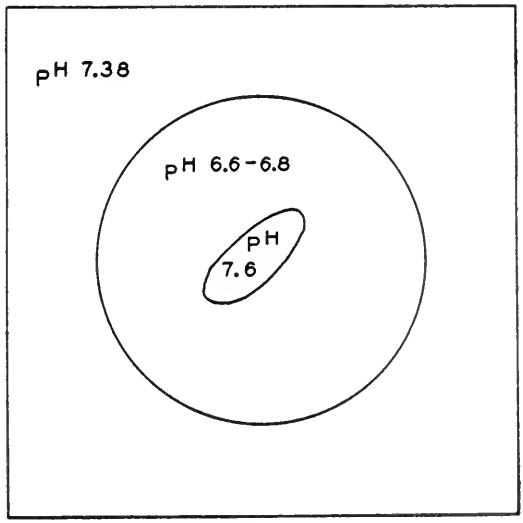


FIG. 2. Diagrammatic model of the cell

Lord Kelvin said in regard to scientific theories: "If I can make a model of it I can try to understand it and if I cannot make a model of it it is difficult for me to understand it". It is possible to make a model of a vital system considering the cell as its unit, but this model is no more accurate than the model of the atom with fixed electrons of Bohr and Lewis in 1918, which has since been developed into the orbits of moving electrons and all their influence upon nuclear physics. Both the living cell and the atom are dynamic systems. Such a model (Fig. 2) for a dynamic vital system can no more be right than a model of the atom with fixed electrons, but it is a step toward understanding and explanation. It merely is a diagrammatic representation for convenience of thought and clarity of consideration.

The cell protoplasm is toward the acid side, surrounded by a boundary or membrane; the cell nucleus is alkaline; the environment, the blood plasma, is slightly alkaline. These changes in acid-base equilibrium (pH) may seem small to the chemist, but they loom large to the student of vital systems where small changes in pH are of great importance, for upon that as one of the factors depends the continuance of the vital equilibrium or balance, the *milieu interne* of Claude Bernard.

Thus, in attempting to explain the action of neutrons upon vital systems, one question must be answered. In what part of the vital system does the effect take place? Is it on the nucleus, the protoplasm, the cell membrane or the environment? If that can be answered the area of research is lessened. In the action of short ultraviolet light, the effect is apparently upon the nucleus. In X-rays' effects, there is no full proof, but it is significant that the effect of X-rays is greater upon those cells having greater amount of cell nuclear elements. This is shown particularly in growing, immature tissue and in certain cancers which are spoken of as "radiosensitive" in that they are more easily affected by X-rays, and have, as a rule, a larger amount of nuclear tissue containing more nucleic acid than normal. Apart from this, there is little evidence as to the site of X-ray effect.

The most significant fact in neutron bombardment is its effect upon growing or immature tissue: this tissue has the largest amount of nuclear elements. This is most marked in the testes, the lymph tissue and the white blood corpuscles. The latter may be considered extremely young tissues, for in man they are renewed at least every twenty-four hours. The normal white blood corpuscle count of man is from 5,000 to 8,000 white cells per cubic millimeter. If the amount of blood in a man of 145 pounds is taken as 6 liters, then 30 billion white blood corpuscles are renewed every day. This seems incredible, yet many things connected with the human body seem incredible.

The number of lymphocytes present in the circulating blood of the cat has been estimated by Sanders, Florey and Barnes (3) at 1250 million. This number, they calculated, was replaced from one-half to almost 3 times every twenty-four hours. For rabbits the daily output of lymphocytes by the thoracic duct was 4×10^9 and the lymphocyte population was replaced with fresh cells about five times daily. The white blood corpuscles have such a short life and are so frequently renewed that they must be considered extremely young, fragile and immature tissues. They must also be present, owing to their short life and quick replacement, in all stages of very rapid growth and development, since their total life is not more than twenty-four hours. They are truly impermanent and ephemeral.

The source of the white blood corpuscles is believed to be in the bone

marrow, the spleen, and lymphatic tissue. By giving a sufficient dose of neutrons it is possible to abolish all white blood corpuscles from the blood, and it may even be possible after a large dose of neutrons to find bone marrow without a single leukocyte. Use is made of this extraordinary fact by taking the level of the white blood cell count as a measure of the amount of injury produced by the neutrons. It is a test easy to do and familiar to laboratory technicians. It is by no means exact because it is influenced by other factors: resistance, age, etc.

The reduction and even disappearance of the white blood cells does not seem to be dependent upon direct killing of these cells, for untreated white blood cells, introduced after exposure to neutrons, live for some time and are not directly killed by repeated radiation. *Something happens which prevents the continued production of these short-lived, easily reconstructed cells.* It is as if a population ceased to be fertile and produced no offspring: they live their life and at the end are not any more.

Such a result in such short-lived cells is significant of the disruption of the processes of their reproduction. If the dose of neutrons is not so great, recovery may take place in time. There is a possibility of the recrudescence of the specific vital factor which is necessary to their continuance.

In consideration of the mechanism of neutron action, it is futile to speak in terms of ionization when there is no idea of where in the vital system that ionization exists. Is it in the nucleus, the cell, the cell boundary, or the membrane? It is true that the greater the amount of neutrons applied the greater the results, but such an amount of energy, hundreds of times that of the short utraviolet line 2536 Å, has comparatively less efficiency as a lethal instrument.

A word should be said about the variability of experimental animals. Chemists and physicists without much experience in animal experimentation are apt to think of them as exact systems, similar to those of the sciences in which they work and in which there are definite quantitative measurements. Not only are different species of animals different in their reactions, but different strains of the same species vary in their reactions. There are strains of mice which have naturally occurring cancer and strains that are cancer-resistant. There are strains of rats that are resistant to transplanted cancer and those that are not. The same is true of many other qualities: age, diet, and even environment may alter their reactions.

Then too, the vital system is a dynamic system: Its chief purpose is life and it goes on living. Alterations in one or other phase will upset the system, but this cannot be explained in terms of such exactitude as exists in chemical and physical experiments. The distinguishing phases of vital systems are that they are provided with the means for the rapid attainment

of equilibrium or vital balance and that they are equipped with highly specific catalysts, enzymes, hormones and vitamins which, in selected chemical reactions, determine the course of the metabolic processes. These catalysts in minute amounts are necessary for the continuance of life and more particularly for the synthesis of rapidly growing cells such as the lymphocytes which disappear after severe neutron bombardment.

In neutron effects, on account of the severity of the result and the quickness of action, vitamins and hormones are less likely to be the deficiency because the effect of their lack may not be shown as quickly as the other neutron effects. The neutron effect may be a two-stage or even a three-stage effect; the altering of these substances may prevent the continuance of the metabolic processes. An example of this is the interference with sulfur metabolism so necessary to growing cells, the chief source of which is methionine, biotin, and thiamine.

The effects of neutrons may be upon one of the enzymes or it may be on the enzyme activator. The substrate may also be affected.

The enzymes, which are all proteins, are dependent upon their structural elements and there is an active catalytic center to involve the combination of the substrate with the active center of the enzyme to form a dissociable enzyme-substrate complex. The substrate is activated and this activated substrate molecule then undergoes rapid reaction with another substance. Both the catalytic activity and the specificity of its actions depend upon the nature of the protein.

Attached to the proteins are prosthetic groups, often of low molecular weight, which combine reversibly with specific proteins, and so undergo rapid oxidation-reduction reactions. The prosthetic group is usually a partner in the chemical reaction catalyzed by the enzyme. The active catalytic center is made up of the structural elements in the protein with which the prosthetic group combines. The enzymes, catalase and peroxidase, are each a complex protein in which a prosthetic group, hemin, is linked to a specific protein. In our research, it has been found that after neutron bombardment, catalase is not affected while peroxidase is much reduced, so that in this case at least the effect is not upon the prosthetic group although in the case of *d*-amino acid oxidase, the inactivation occurs in the prosthetic group, alloxine adenine nucleotide.

The prominent reactions of the cell are the processes of hydrolysis and condensation, and of oxidation-reduction. Organic substances may undergo other reactions such as amination and deamination, alkylation, etc., and these go on continuously and together. Characteristic of life is the large molecule such as protein, easily destroyed and easily reconstructed. Synthetic reactions require energy for their efforts and so they must be coupled with energy-producing reactions. The steady state, the vital

balance, or *milieu interne*, represents a dynamic equilibrium in which enzymes are continuously catalyzing not only degradation and regeneration reactions, but also chemical reactions to make available for synthetic processes the energy acquired from the environment. The vital balance is determined, not only by the thermo-dynamic potentials, but also by the kinetics of the individual reactions catalyzed by the enzymes.

Very minute particles often play an extraordinarily large part in the metabolism of living tissue. For example, the growth hormone from the pituitary recently discovered by Evans of California is so powerful that ten gammas, ten-millionth of a gram, is sufficient to bring about a gain of weight of one gram a day in rats whose pituitary gland has been removed. This is only one example of the power which certain substances in very minute amounts have upon growth and reproduction of cells.

If the popular explanation of the physicists of absorption of quanta is to be made intelligible, it must be considered where, in the cell system, this absorption takes place. To take the homely metaphor of the automobile, if a bullet is fired from a rifle and hits the machine, the force is absorbed; but if it is absorbed on the fender, it does little harm and if it is absorbed on the timer, the machine is dead. How much more complicated and how much less understood is the dynamic cell system. If the quanta are absorbed on Evans' growth hormone, as quite well might be possible, then growth stops as do the white blood cells after neutron bombardment. It may be the destruction of the sensitive entity which prevents the growth and reduplication of cells. The mode of destruction might quite well be in a breakage of the weaker molecular linkages of the molecules concerned as seems to be the case in the effects from shorter ultraviolet light from 2654 Å to 2536 Å.

With the background of our previous work on the treatment of cells by shorter ultraviolet light and the absorption of these wave lengths by the nucleic acid of the cells, it was natural to begin studies in regard to enzymes concerned, particularly desoxyribonuclease, but this enzyme was shown not to be affected by neutron bombardment. Recent work by Dale, Meredith and Tweedie (4), however, has shown that in certain enzymes there is a protective or indirect action from additional substances in the solution capable of reacting with the intermediate products and so diverting some of the radiation to itself. The effect of X-rays on aqueous solutions of enzymes has been frequently interpreted in terms of the "quantum hit" theory without proper consideration of the indirect action. The effect depends upon the concentration and purity of the enzyme solutions and so shows reactions which would not be present in the vital systems owing to protective substances. This complicates the matter. Radiation produces a smaller effect with the added protective substances which are often too

small to absorb radiation directly to any appreciable extent but the molecules take a share in the radiation product and thus the number of molecules in the main solute inactivated is reduced. So that the evidence of the effects of radiation upon pure enzyme solutes cannot now be taken at face value.

Recent and very interesting work by Mitchell upon X-rays (5) much along the same lines as our own, in regard to the effects of radiation upon the nucleic acid of the cell nucleus, comes to the conclusion "Evaluation of the ionic efficiencies shows that significant disturbances of both carbohydrate and nucleic acid metabolism are produced by therapeutic doses of X and gamma radiations, probably by means of enzyme inactivation". After radiation in dividing cells he believes there is, due to mitotic inhibition, no significant increase in the thymonucleic acid synthesized by the nucleus. This histochemical evidence suggests that the small increase in nuclear absorption observed in some cases after radiation is due to an increase in pentose nucleotides either formed within the nucleus or diffused into it from the protoplasm. In our work upon neutrons, it has been considered that nucleic acid which is of large molecular weight, when broken into smaller molecular weight, pentose nucleotides, may diffuse when the larger molecular weight substance could not and that these nucleotides will inhibit the action of the enzyme ribonuclease to inhibit the synthesis of nucleic acid so necessary to mitosis and cell division. This work is continuing and gives an interesting area of research into neutron mechanism.

Chemical processes, and life is a chemical phenomenon, are involved in the immediate effect of ionizing radiation. This is apparently true in regard to the turnover of desoxyribonucleic acid and Hevesy (6) has shown that after radiation with 1000 r of X-rays the turnover of phosphatide is also diminished from the nuclei and from the cytoplasm less so. This is of interest because of the importance of desoxyribonucleic acid phosphorus and phosphatide phosphorus in the nuclei of dividing cells.

One theory in regard to radiation effects is that there exists in the cell a special sensitive volume within which ionizations are biologically effective and that these account for subsequent changes. More than one ionization may be required to produce a biological effect but any ionization which occurs within the cell, but outside the sensitive volume, is ineffective. This view of the mode of action of radiation has become known as the "quantum hit" or target theory. Differences in sensitivity to radiation are explained by the chance distribution of ionization in the vital volume of the cell system.

An alternative hypothesis is that chemical or metabolic changes are produced in the cell (or in the environment ?) by irradiation and that the

biological results of physical as well as chemical agents may be explained upon the assumption that individual cells differ in the reactions and in the changes produced. The weakest (most radiosensitive) succumb first, then the less weak and the strongest (most radioresistant) the last of all.

The target theory cannot be made to fit all types of biologic response since by definition it made no allowance for adaptability in living organisms. The cell (and to speak of the cell must be to include the cell system with its environment) is not inert until it is dead and it is capable of recovery if it is not hurt too much: so long as it is alive it is capable of recovery. The types of response to radiation must be learned from observation under different biological conditions and the same cell may differ in its susceptibility to radiation under different conditions, as for example, dryness, metabolic activity, stage of growth and its age. There is a danger in attempting too much simplification by physical explanations in such a complex biological matter with its delicately poised and elaborate mechanism of the cell system.

The effect upon chromosomes in production of mutations in radiosensitive organisms, as drosophila, is an argument against the target theory, since not all cells are equally susceptible to mutational effects of radiation but are also affected by temperature, nutrition, anesthesia and degree of germination. Environmental conditions alter the degree of response.

The number of chromosome breaks produced are proportional to the amount of the dose but independent of intensity (7, 8, 9), but neutrons are more efficient in producing breaks than are X-rays. Mutation effects are dependent upon the wave length, independent of intensity, but general biological effects vary with alteration of the intensity and the wavelength. These observations are explained on the hypothesis that a chromosome is broken by the passage through it of a single ionizing particle, but that it is necessary for the ionizing particle to be sufficiently densely ionizing for several ionizations to be produced near the chromosome. But this still gives no explanation as to what processes or chemical reactions are produced to cause the break in the chromosomes.

Radiation has a marked effect in interfering with cell proliferation and the dose which produces the first recognizable changes in cell proliferation is always small relative to the direct lethal dose for the same tissue. During development radiosensitivity decreases as the age increases, but the decrease is not necessarily progressive throughout development. It is a mistake to consider all cells in the body as of the same age. The frequently renewed white blood cells, for example, are young cells without much differentiation and differentiation reduces radiosensitivity

Apart from a direct lethal effect, cells may be so injured by radiation as to be incapable of successful division and may thus either perish in at-

tempting mitosis or produce non-viable daughter cells. In cancer it is obvious that, if sterilization of all potential dividing tumor cells could be achieved, their total destruction by radiation would be unnecessary, since the altered cells would gradually disappear due to their short life. Like weeds in a field of wheat, if prevented from multiplying without injury to the grain, the weeds will die out and it is unnecessary to kill both weeds and grain together.

Measurements of the biological action of X-rays and of γ-rays show that living cells vary in their response to irradiation. Differentiated cells are, in general, more radioresistant while dividing cells, immature cells, are more generally radiosensitive, and these are more sensitive at certain stages of their division cycle than at other stages. There is also variation in cells of a homogeneous population and this has been put forward as an evidence of individual variation and, on the contrary, as an expression of the relationship between the dose of radiation and the percentage of the cells which have received by chance a number of quantum hits to produce death, the "quantum hit" or target theory.

According to the first theory, when a group of cells is irradiated the radiant energy is equal in amount and is similarly distributed in each individual, whereas the capacity to resist the absorbed energy is different in different individuals. According to the second theory, the energy which is absorbed by cells or by specially vulnerable parts of cells is different in different individuals, whereas all the individuals have an equal capacity to resist a given injury.

The quantum hit theory assumes that the resistance of all individual cells to a given amount of absorbed energy is the same. The energy is absorbed in quanta and presumably the quantal absorption will have a random distribution amongst the different cells. Some have calculated that the number of quanta absorbed in the cell must be great if a lethal action is to occur and the quantum hit theory has been modified accordingly. The present form of the quantum hit theory is that a lethal number of quanta are absorbed in a particular "sensitive spot" in the cell and this causes its effect. It is as if a duck were shot with a large number of small shot and only one or a few struck the brain or another mortal part to produce death, while the other pellets were absorbed, but not to produce lethal effects.

Individual variation is a characteristic biological phenomenon and, no matter what care is taken to achieve homogeneity, the occurrence of individual variation can be proved in respect to every property of individual cells that is capable of measurement. It is reasonable to assume that in all living cells, the resistance to injury varies. The difficulty in this assumption of variation is the absence of any plausible explanation as to how this variation, susceptibility or resistance to radiation is brought about.

The survival of some cells after a large dose of radiation may be due to the capacity to repair injuries which is different in different organisms or the radiation quanta may not affect certain of the cells in their sensitive spot. Usually organisms irradiated with a lethal dose survive for a period and then die. The tissues are capable of repairing the injury produced when the dose is small and so it may be counteracted, but when the dose is large enough, the repair processes produce a negligible effect upon the total amount of injury, so that at high intensities, the biological effect of radiation is independent of the intensity.

In all these considerations of radiation effects which are those of X-rays and γ-rays, the cell is chiefly imagined to be a fixed organization with uniform structure. Nothing is farther from the truth. It is a complicated, dynamic system with many contributing factors which are necessary to its life, and these are in delicate balance or equilibrium. To speak glibly of absorpton of quanta of energy by the cell is like saying another dynamic system, your automobile, won't run because it has absorbed some energy due to an injury. Its defect may occur in dozens of places and, as we know more about the mechanism of the automobile than we do of that more complicated, dynamic system, the cell, we can explain the course of the injury to it. We can then find a cracked spark-plug or some other defect, but as to the cell, we don't know enough about the mechanism to say what happens, and to say that energy quanta are absorbed only befogs the question by giving a name to a process which is not understood, and, if repeated enough, has the effect of obscuring speculation into the mechanism.

Certain generalization, however, may be made. Immature cells and dividing cells are more easily affected by radiations in general. The effect upon the chromosomes is striking, but it may be only one of the ways in which biologic material responds to radiation. Muller himself warned that "not all the effects of radiation in killing organisms or disturbing their development are referable to changes either of the class of gene mutations or chromosome re-arrangements".

On highly differentiated cells the effect of injury from radiation is of such character as that resulting from other injuries or chemical poisons. When the blood supply is restricted or inhibited by radiation, it has even been suggested that radiation effects on a complex tissue are the results of the action on the circulation, but later studies disproved this. For the pathologist to reconstruct the mechanism of action of radiation from the dead, fixed tissue under the microscope is impossible, and the pathologic picture is in no fashion specific, for it can be produced by other injury or poisons.

The lymphocytes of the blood are highly susceptible to radiation, and if a very large neutron dose is given they disappear; with a smaller dose some of them disappear; if the dose is not great they recover their former num-

bers. Is this recovery due to repair processes on some injured cells or have enough of the mother cells escaped to make a nucleus of a new accretion. If they were a flock of ducks subjected to a blaze of gunshot, some may be injured to recover later; some may have escaped the gunfire completely to form the nucleus of a new flock. The explanation escapes us: it may be one or both.

At various dose levels a change in behavior occurs in irradiated cells. At the highest dose level the result is quick death, presumably by severe breakdown in the physicochemical system of the protoplasmic cells; at lower levels, death results but a longer time is required for the upset of the machinery of the dynamic system of the cell. The changes may be transitory and reversible or they may be permanent where the radiation injury disappears but leaves the tissue in a state of lowered resistance to further radiation. There is no single type of response which can be called the characteristic effect of radiation.

Much has recently been said about the great value that the atomic bomb discovery will be to the study of cancer, but does it really promise more than the discovery of other destructive forces? Its only present promise is to popularize the use as "tracers" of radioactive material which we have been producing by the cyclotron for eight years at least. Radioactive sulfur and radioactive phosphorus, the ones most useful in biological study, are easy to produce; radio-carbon is more difficult. To use radioactive tracers merely to tell by Geiger counters into what organ the radioactive substance goes, gives little information; almost each experiment with radiotracers requires a special chemical technique in addition to get real information.

Radio-phosphorus has been produced by the cyclotron and used in the treatment of leukemia and polycythemia for a number of years with considerable success. Its effects upon cancer have not been encouraging. Radio-iodine produced by the cyclotron has been also used in the treatment of certain forms of goiter with considerable effect. The atomic pile may produce these substances, but they are not new.

The real value of the new interest in radiation effects is the effort to explain their action in stopping the growth and division of young, immature cells. Cancer is a problem in cell division and cancer cells are relatively immature rapidly-dividing cells. If their division can be inhibited, their relatively short life and weakness will cause them to die and, if no new cells are formed, the cancer will diminish and disappear. If the neutron radiation study shows the mechanism whereby lymphocytes are prevented from reproduction, the mode of prevention of cancer cell division is at hand. It may then be possible to apply and produce these conditions within the cancer cells, even without radiation, and so produce a stoppage of cell division and growth.—And that is our aim and hope.

REFERENCES

(1) Allen, A. J., Franklin, R., and McDonald, E., *J. Franklin Inst.*, **218,** 701 (1934).
(2) Ely, J. O., and Ross, M. H., *J. Franklin Inst.*, **242,** 85 (1946).
(3) Sanders, A. G., Florey, H. W., and Barnes, J. M., *Brit. J. Exptl. Path.*, **21,** 254 (1940).
(4) Dale, W. M., Meredith, W. J., and Tweedie, M. C. K., *Nature*, **151,** 280 (1943).
(5) Mitchell, J. S., *Brit. J. Radiol.*, **16,** 339 (1943).
(6) Hevesy, G., *Nature*, **158,** 268 (1946).
(7) Lea, D. E., and Catcheside, D. G., *J. Genetics*, **44,** 216 (1942).
(8) Thoday, J. M., *J. Genetics*, **43,** 189 (1942).
(9) Giles, N., *Genetics*, **28,** 398 (1943).

Chapter 2

COMPARISON OF PHYSICAL PROPERTIES OF FAST NEUTRONS WITH THOSE OF SOME OTHER FORMS OF RADIATION

By T. ENNS

A. Ultraviolet rays are electromagnetic waves travelling at the velocity of light. The photon energy* is several electron volts. For any homogeneous material placed in their path, electromagnetic waves are absorbed exponentially: if a given thickness of material absorbs half of the incident radiation, an additional equal thickness of material absorbs half of the remaining radiation. However, the relative absorption differs greatly with the chemical composition of the absorber, as the energy of the photons falls within the range of organic and inorganic molecular binding energies. For the same reason the relative absorption differs greatly when the photon energy (and hence the wave length) of the incident light is changed.

B. X-rays and gamma rays are also electromagnetic waves, but have photon energies much greater than those of ultraviolet rays. High energy rays may have photon energies of several million electron volts. In recent terminology the names X-rays and gamma rays more often indicate the source of radiation rather than its nature. In general, gamma rays have a lower energy limit of approximately 100,000 electron volts. X-ray absorption, for energies below 100,000 electron volts, depends on the exact energy as well as the elements (but not the chemical composition) of the absorber. The absorption of higher energy X-rays and gamma rays depends almost entirely on density of the material and radiation energy. Radiation of higher energy requires more material to effect a given reduction in intensity. The processes of absorption are quite complex, but most of the energy is lost in the production of X-rays and electrons of lower energies which in turn produce electrons and ions of still lower energies, the process being continued down to energies of one volt or less.

C. Beta radiation consists of beta particles. These may be either electrons or positrons. Their rest mass is 9×10^{-28} grams and their velocity increases with their energy, but is always less than the velocity of light. Beta particle spectra from radioactive elements have peak energies between 0.05 and 20 million electron volts. Beta particles are not absorbed exponentially as is electromagnetic radiation, but have a definite absorber range beyond which no beta radiation may be detected. This range in-

* Photon energy in volts $= \frac{12343}{\lambda}$ where λ is the wave length in Å.

creases with particle energy, and depends almost entirely on absorber density. For high energy beta radiation the range is several grams per sq. cm. of absorbing material.

The products of beta particle absorption are chiefly electrons of lower energy, which in turn produce electrons and ions of successively lower energies down to magnitudes of one electron volt or fraction thereof.

Positrons also produce gamma rays of 511,000 electron volts (two quanta per particle).

D. Alpha radiation consists of alpha particles (helium nuclei—hence doubly ionized helium). These particles have a mass of 6.6×10^{-24} grams. The term is generally applied to high energy particles (4 to 7 million volts) of the radioactive elements. These particles have a very short range, but produce heavy ionization along their path. The specific ionization is almost constant along the path of the particles, and much heavier than that along the path of X-rays or beta particles.

E. Neutrons are uncharged particles with a unit mass of 1.66×10^{-24} grams. Fast neutrons are generally considered to be those with energies from 0.1 to approximately 15 million volts. As neutrons have no charge, they can lose energy only by direct impact on nuclei. High energy neutrons pass through considerable masses of material without much absorption or energy loss. Their penetration is comparable to that of very high energy X-rays or gamma rays. However, the nature of neutron absorption differs radically from the absorption of X (and gamma) rays. While the absorption of X-rays increases with density of the absorber, that of neutrons increases with density of nuclei in the absorber. Materials such as water and paraffin with their large concentration of hydrogen nuclei are good neutron absorbers.

When fast neutrons lose some of their energy in a relatively thin absorber, they do so mainly by the production of recoil nuclei. These are charged nuclei of the irradiated material which have received a large portion of the energy of the incident neutron. The recoil nuclei in turn produce heavy ionization along their short paths. In animal tissues, these nuclei are mainly hydrogen, oxygen, and carbon ions. Their behavior is therefore comparable to that of alpha particles. In materials predominating in heavy elements, the recoil nuclei will be heavier and have shorter paths with denser specific ionization.

Chapter 3

FAST NEUTRON IRRADIATION PROCEDURE

By T. ENNS, H. M. TERRILL, and JAMES M. GARNER, Jr.

The cyclotron was used as the source of fast neutron radiation. The neutrons were produced by deuteron bombardment of beryllium inside the cyclotron chamber. Location of the cyclotron and target are shown in Fig. 1. The numbered rectangles represent positions for which relative dosage values were determined.

To obtain simultaneous exposures of animals of high and low level dosage groups, cages were placed in different positions. Cages placed nearer the target received radiation dosage at a higher rate than more distant cages.

IRRADIATION

Radiation Cages. The cages, shown in cross section in Fig. 2, were designed so that the animals were completely surrounded by one-inch thickness of lead, with an additional two inches of lead between them and the target. The cages were lined with sheet copper. In the front wall of the cage was an additional sheet of copper, with many perforations. Provision was made for introducing a stream of fresh air between this sheet and the lining. This air flow was turned on whenever animals occupied the cages. There was one inch of clear space all around between the cage and the overlapping cover.

The cage temperature was approximately room temperature. Cyclotron operation produced no temperature rise in the cyclotron room. The great heat capacity of the cage metal as well as the circulating air prevented any great temperature rise in the cages due to the presence of the animals.

Cages 4, 5 and 6 each held 2 dogs, 4 rabbits or 24 rats at one time. Cage 7 was considerably smaller, having a capacity of only one rabbit or ten rats.

Procedure. Dogs were placed directly in the cages without any separate container. They were faced east and west on alternate days. The space available for each dog was $25'' \times 8\frac{1}{2}'' \times 10''$.

Rabbits were placed for irradiation in individual copper boxes measuring $5'' \times 9'' \times 12\frac{1}{4}''$ (Fig. 3). These boxes had a handle on one side for convenience in loading and unloading, and were perforated for ventilation near the end adjacent to the animal's head. The boxes were symmetrical at top and bottom and were turned upside down on alternate days if animals were irradiated on more than one day. They were labeled M W F on one edge and T Th S on the opposite edge. Thus, on Mondays, Wednesdays and

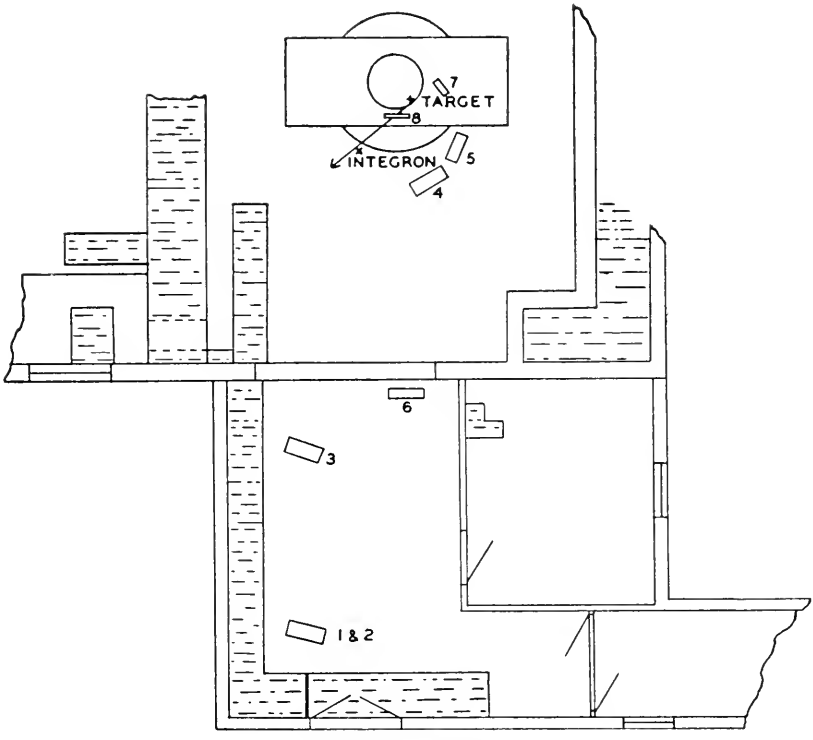


FIG. 1. Plan of radiation area

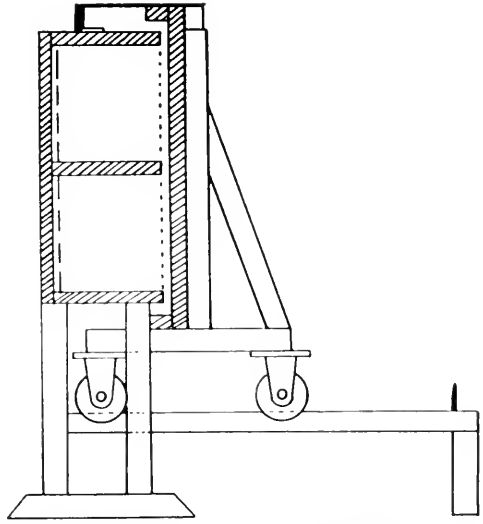


FIG. 2. Cross section of treatment cage (½" = 1")

Fridays, the M W F side was placed uppermost while on Tuesdays, Thursdays and Saturdays, the other side was turned upward. Since the handle was always turned toward the rear, the animals faced alternately east and west.

Rats were placed in copper boxes, each of which held twelve individuals. These boxes measured $9\frac{1}{2}''$ x $2\frac{3}{4}''$ x $24\frac{1}{2}''$ on the outside, and each rat had a space of $2\frac{5}{8}''$ x $2\frac{3}{8}''$ x $8\frac{3}{8}''$. The boxes were ventilated front and rear.

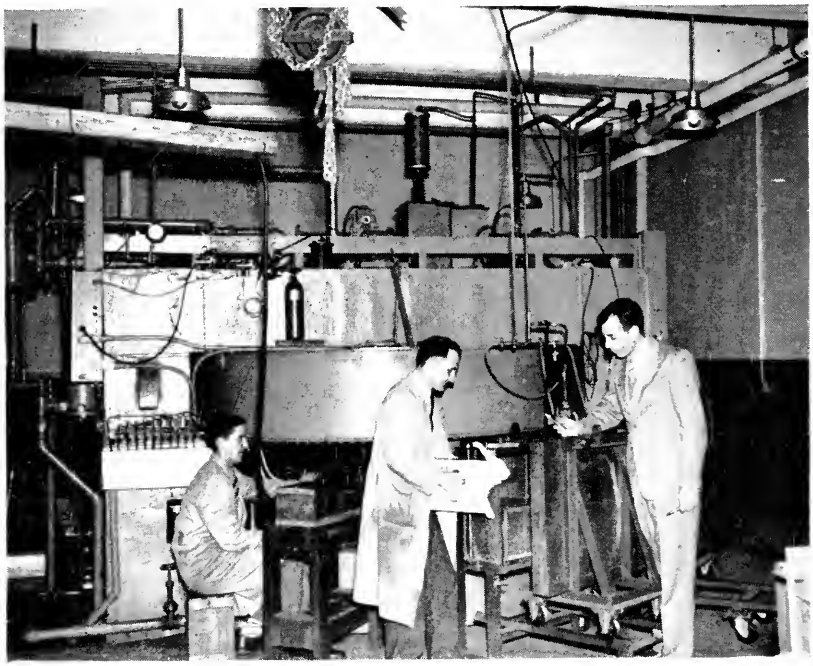


FIG. 3. Irradiation procedure

Mice were irradiated in cage 7. They were placed in two rat boxes, 15 mice per box. The boxes were located one above the other.

The lead cylinders used in corn irradiation were placed in position 8. Bacteria were irradiated in the same general region.

NEUTRON ENERGY

The deuteron beam energy was 9.5 million electron volts. The beam direction is indicated by the arrow in Fig. 1. The neutrons were produced by the reaction

$$Be^{9} + H^{2} \rightarrow B^{10} + N^{0}.$$

The maximum neutron energy was 13.5 million electron volts, these being emitted from the target in the direction of the incident deuterons (arrow in Fig. 1). The main intensity of radiation emitted in this direction was due to neutrons of approximately 6 million electron volts energy. This was deduced from the energy determinations of Aebersold and Anslow (1) for a similar neutron beam, and from comparison with the neutron spectrum reported by Staub and Stephens (2).

Positions 1 to 6 were as close to the line of maximum energy neutrons as room dimensions permitted. For positions 1, 2, 3, 4 and 6 the deviation was not sufficient to decrease the energy of the peak neutron radiation intensity. Position 7 was almost directly behind the target. The cage in this position received neutrons with a main intensity lower than 6 million electron volts but not lower than 2 million electron volts. It did not, however, receive any greater proportion of slow neutrons than the cage in position 4. Cage 5 received a main neutron radiation intensity lower than that of cage 4 but higher than that of cage 7. The energy of the main intensity neutrons was estimated as 3 million electron volts for position 7 and 5 million electron volts for position 5. Care was also taken to arrange the cages so that no cage was interposed between the Be target and any other cage.

As positions 3 and 2 were near the water walls, they received thermal (very slow) neutron radiation formed by slowing down of fast neutrons in the water walls. These neutrons were removed by covering the water walls with cadmium absorbers.

The neutron intensity spectra in positions 4, 3, and 2 were compared. This was done by surrounding a .25 r Victoreen ionization chamber by successively thicker layers of paraffin and measuring the amounts of chamber discharge caused by equal neutron dosages emitted by the cyclotron. The results are shown graphically in Fig. 4.

Fast neutrons striking the paraffin are slowed and deflected so that they produce an increase of ions in the chamber. A peak intensity is reached when sufficient paraffin has been used to slow down all the fast neutrons. Further addition of paraffin cuts down the neutron intensity as the extra paraffin absorbs the slow neutrons. Hence the paraffin thickness at which maximum intensity occurs is greater if fast neutrons predominate in the radiation field.

Measurements made before the installation of the cadmium are shown in Fig. 5. Here cage 2 has a maximum intensity point for a very thin paraffin absorber, indicating a high slow neutron intensity previous to installation of the cadmium shielding.

The curves of Fig. 4 are approximately identical. Position 4 was close to the target and presumably received most of its neutron radiation in-

tensity from 6 million electron volt neutrons. Hence positions 3 and 2 received neutron radiation with a maximum intensity of approximately he same energy.

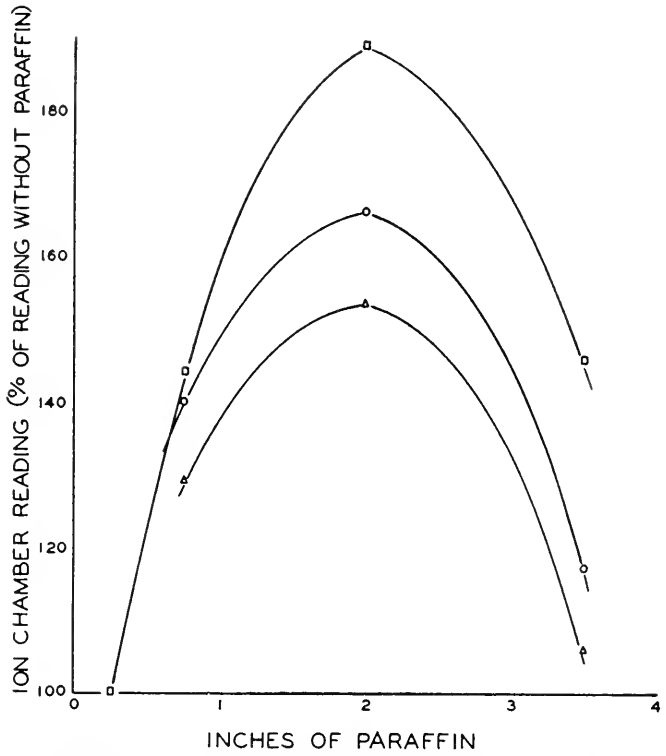


FIG. 4. Comparison of the neutron intensity spectra in positions 4, 3 and 2. □ = Position #4. ○ = Position #3. △ = Position #2.

NEUTRON RADIATION INTENSITY

The method of intensity measurement followed that of Aebersold and Lawrence (3). There is at present no well-defined standard of fast neutron radiation intensity. The method of Aebersold and Lawrence seemed the most practical and, as we worked with a neutron spectrum similar to theirs, a comparison of results was easier. An important advantage in this method is that all radiation intensity measurements are made with a Victoreen 100 r chamber. These chambers are commonly used in X-ray work and are readily available.

The Victoreen chamber gives its reading in r (Roentgen) units. We have followed the practice of Aebersold and Lawrence and called these readings n (for neutron radiation intensity) units. This was done for two

reasons. The Roentgen refers only to X (Roentgen) radiation. The biological effect of an n unit of neutrons is different from that of an r unit of X-rays even though both give the same reading when measured with a Victoreen 100 r chamber.

The dosages were controlled by a Victoreen Integron. This instrument consists of ionization chamber, amplifier, and meter. The meter indicates the chamber charge in r units and may be set at any dosage value. The

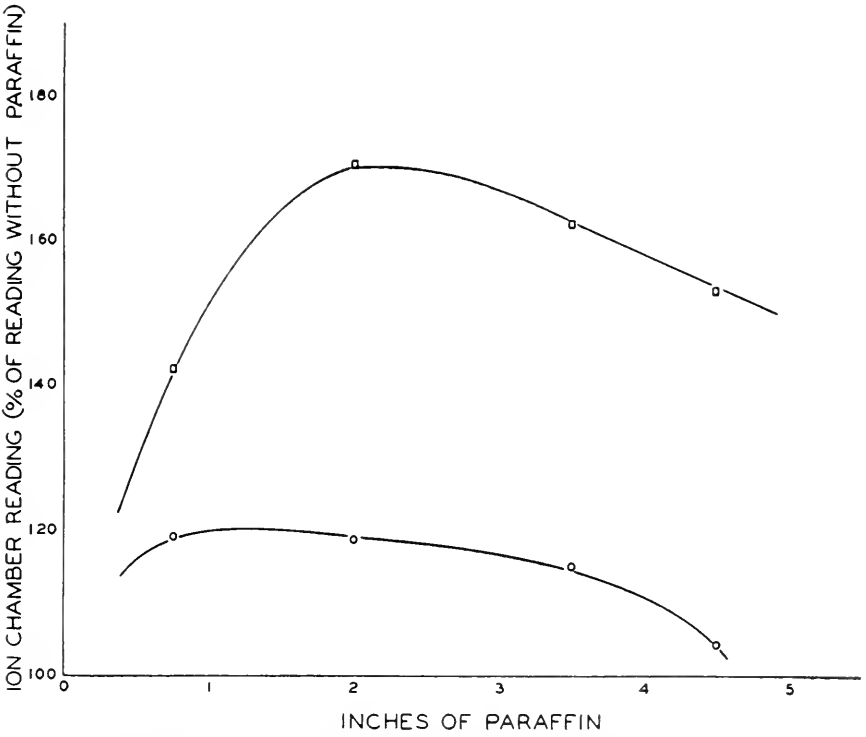


FIG. 5. Neutron intensity previous to the installation of the cadmium shielding. □ = Position #4. ○ = Position #2.

reading decreases as the ionization chamber is exposed to radiation until the reading becomes zero. Thus, if the chamber is to receive 10 r, the meter is set at 10 r and the chamber is exposed. When the chamber has received the 10 r, the meter reads zero and closes a relay which rings a bell and lights a light. This relay is connected to the cyclotron oscillator in such a way that it turns off the oscillator and hence stops radiation when the exposure is completed and the meter reads zero.

It was impractical to mount the Integron ion chamber in the cages, so it was placed in the position indicated in Fig. 1. Like the cages, it was

surrounded by one inch of lead. Four 100-r chambers were placed in cage 4 and their discharge was compared with the integron discharge reading. In successive trials the integron position was changed until a discharge of 29.3 divisions (full scale reading is 30 divisions) corresponded to a mean dosage of 1.7 n in position 4.

This relationship of integron discharge to cage 4 was checked three times in the course of a year.

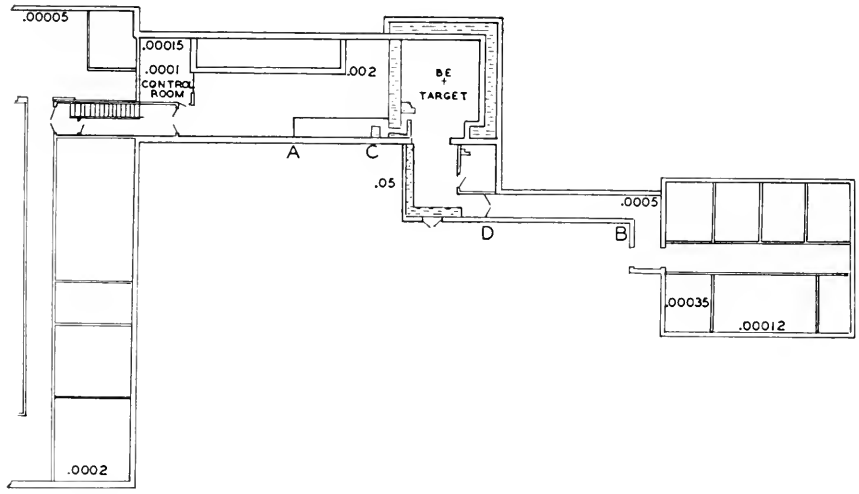


FIG. 6. Plan of the cyclotron room

The 100 r chamber readings corresponding to a 29.3 division discharge of the integron are given below:

	Total of Chamber Readings	Total Integron Divisions
Measurement of 3–27–45	274.5 n	29.3 × 160
Measurement of 12–27–45	63.8 n	29.3 × 40
Measurement of 4–2–46	102.7 n	29.3 × 60
	Total = 441.0	Total = 29.3 × 260

These measurements give a mean position-4-dosage of 1.7 n for a 29.3 division discharge of the integron.

Dosages for positions 5, 6, and 7 were determined directly by placing four 100-r Victoreen ion chambers in the cages. The mean values obtained for different segments of the cages are recorded below in terms of the position-4-dosage obtained during the same exposure time:

	Cage 4	Cage 5	Cage 6	Cage 7
Ratio of dosage	100	63.2	12.6	395
Dosage for 29.3 integron divisions	1.7 n	1.07 n	0.21 n	6.72 n

PROTECTION OF PERSONNEL

The cyclotron was provided with both earth and water shielding, and was located at some distance from the other laboratories. The main water walls were three feet thick and the roof was covered with two feet of water. Depression of the cyclotron room floor level placed the plane of the beam and hence the plane of maximum radiation just below the floor level of the main building. The long duration and high intensity of neutron radiation in this program made it necessary to evaluate all this protection. It was also essential that provisions be made against accidental entry into the radiation area.

A plan of the cyclotron room, animal house (right) and part of the main building (left) is shown in Fig. 6. The neutron radiation dosage in "n" units for normal daily operation (6 days per week) is shown by the numbers in the area where measurements were made. This dosage was exceeded during the last year of the program but did not average double the earlier dosage. Thus *doubling* the dosage values given in the diagram gives the maximum average daily dosages at any of the points.

The point *A* represents the point of closest approach to the radiation area by cyclotron operators during normal operation. *C* is the limit of permitted approach during radiation. *B* is the limit of approach permitted in the animal house. Illuminated warning signs at *C* and *D* automatically went on during cyclotron operation. An additional safety device was a photoelectric cell installed at *D* which operated a bell signal in the control room so that the operator could stop irradiation if the cyclotron room was inadvertently entered from the animal house.

With reasonable care by the cyclotron operators it was thus impossible for anyone connected with the radiation program to receive an accumulative dosage of more than .001 n per day. Actual dosages received by personnel were probably a fraction of this. Blood examinations were made periodically upon all operators and attendants who might be exposed to radiations.

Additional radiation monitoring was achieved by film badges worn by personnel throughout the program. These consisted of dental films set in brass frames. A portion of the film was covered with a cadmium shield to absorb neutrons, this area being used to monitor gamma radiation only. These badges were checked weekly and made possible detection of any accidental entry into the radiation area. During the program there was no evidence of any badge being exposed to detectable amounts of radiation while worn by personnel.

REFERENCES

(1) Aebersold, P. C., and Anslow, G. A., *Phys. Rev.*, **69**, 1, 1946.
(2) Staub, H., and Stephens, W. E., *Phys. Rev.*, **55**, 131, 1939.
(3) Aebersold, P. C., and Lawrence, J. H., "Annual Review of Physiology", Stanford University, **4**, 25, 1942.

CHAPTER 4

RELATION BETWEEN NEUTRON DOSE AND THE MORTALITY, BODY WEIGHT AND HEMATOLOGY OF WHITE RATS

BY JAMES L. LEITCH

INTRODUCTION

For the past ten years, there has been much interest concerning the action of neutrons on organisms and biological systems. Much of the earlier work to 1944 has been summarized recently by Stone (1). In general, the effects produced by neutrons have been found to be qualitatively comparable to those produced by X-rays. For equivalent effects, however, Lawrence (2) reports that the X-ray dose varies from 2.5 to 10 times that of the corresponding neutron dose when both are measured in a Victoreen r chamber. However, there are little or no specific data concerning the mechanism of action of neutron rays on biological systems as is also true of X-rays. Other than the fact that the ionization produced by both these types of radiations is responsible in some manner for their effects, nothing definite is known concerning how such ionization in tissues and other biological systems produces the observed changes. Before a detailed study of the mechanism of action of neutrons on biological systems could be initiated, it was deemed advisable to make a fairly comprehensive study of the relationships existing between the neutron dose in whole-body irradiation and the various biological effects such as leukopenia, loss of body weight, erythropenia, etc., comparable to that made by Henshaw (3) on Roentgen injuries.

Lawrence and Lawrence (4) found that for albino rats (2.5 to 3 months old) of the Wistar Strain neutron doses of 14 to 42 r produced a decrease in leukocytes together with an increasing proportion of polymorphonuclear leukocytes which became greater as the dose was increased. In this range, the rats showed a definite leukopenia with recovery without showing any signs of illness. Two rats of this age-group died: one on the 11th day after 72 r with an increase in the red blood cell count from 9.60×10^6 up to 13.00×10^6 and the second on the 9th day after 160 r. Just prior to death both of these rats showed a white blood cell count increasing after a minimum on about the third day together with necrotizing lesions of the head, the latter being due probably to secondary infection. On the 5th day, both of these animals were obviously sick with rough fur and arched back, did not eat or drink and lost weight.

Lawrence and Tennant (5), using Swiss mice, 6 to 8 weeks old, found the following relationship between neutron dose and mortality:

Neutron Dose *r*	Mortality Ratio	Time of Death *day*
133	5/15	10th to 28th
147	6/15	8th to 18th
159	7/15	4th to 13th
168	11/15	8th to 42nd
179	10/15	3rd to 43rd
194	10/15	3rd to 46th
207	13/15	3rd to 15th
223	15/15	Average of 6.9
245	15/15	Average of 5.4
298	15/15	Average of 3.5

They conclude on the basis of gross pathology that the mucosa of the small intestine and the lymphoid and hematopoietic systems were the most sensitive to irradiation and that the mechanism of death for both X-rays and neutrons is a combination of tissue destruction and enterogenous infection. The former effect predominated in acute deaths following large doses.

Yamashita (6) studied the effects of neutrons on young rats weighing from 14 to 30 grams. After 30.8 r, the body weight decreased continuously until death on the 11th to 17th day. Following 26 r, the body weight remained stationary for two weeks while after 12 r there was an increase after one week, but the growth rate was still very poor after four weeks. The leukocytes decreased as also did the proportion of lymphocytes even at 12 r and remained low for 17 days. Yamashita also reports that the red blood cells and blood hemoglobin gradually decreased and that in some cases nucleated red blood cells appeared in the peripheral blood.

Since only a small amount of data is available in the literature on whole-body irradiation with neutrons involving a wide variety of conditions, no definite conclusions may, at present, be made concerning the relationship between dose and effect. In general, however, decreased body weight, leukopenia, and possibly erythropenia are associated with irradiation with relatively heavy doses of neutrons. In the work of Lawrence and Lawrence (4) and Lawrence and Tennant (5), the neutron beam contained some gamma rays, the effects of which were considered by these authors as insignificant in comparison with those of neutrons. Only in the results of Yamashita (6) can the effects be attributed to neutrons alone since he used a deuteron-deuteron reaction for the production of the neutrons in which no gamma radiation is formed.

In the experiments to be reported, proper shielding eliminated gamma rays (Enns *et al.*, 7) so that the radiation affecting the rats was composed

entirely of neutrons. Furthermore, one very important phase of the problem which has not previously been reported is that of the effect of repeated exposure to relatively low doses of neutrons. A knowledge of the effects of repeated exposures to low neutron doses is of importance to those working with neutrons in order that protective measures may be instituted where necessary. The experimental work described in this paper shows the general effects of short exposures to relatively heavy doses of neutrons as well as those of repeated exposures to low doses.

EXPERIMENTAL DATA

General Experimental Data. All experiments described in this paper were carried out on white rats of the Brooklyn Strain from the laboratory colony. Adult rats weighing from 150 to 220 grams were chosen for this work since it is planned to use animals of this size in future detailed investigations of the mechanism of action of neutron rays. Prior to exposure to irradiation the rats were kept under observation for at least two weeks during which time repeated checks of body weight and white blood cell counts were made and also in some instances red blood cell counts and blood hemoglobin determinations. This latter determination was carried out by the acid-hematin method on .01 ml. of blood using a Klett-Summerson photoelectric colorimeter with a No. 42 filter. Control groups of rats of comparable weight were also studied along with the irradiated groups. Identification of tumors and histological studies were made by Dr. Douglas M. Gay of this Laboratory.

All animals were irradiated by the cyclotron of the Biochemical Research Foundation under the supervision of the Physics Department. The details of this cyclotron, of the methods and conditions of irradiation, and of the characteristics of the neutron beam have been described by Enns *et al.* (7). All neutron doses have been reported in terms of n units, measured on the 100 r Victoreen chamber. A summary of the radiation data for all experiments is given in Table I.

In subsequent sections of this paper, the experimental data have been divided into three groups for discussion purposes as follows:

1. Neutron doses of 17.5 to 240 n.
2. Neutron dose of 10 n repeated twelve times—Accumulated dose of 120 n given over a period of 14 days with no irradiation on the third and fourth days.
3. Neutron doses of 1.8 n repeated—Given daily, except Sundays, for a total of 251 doses (total elapsed time of 299 days) in one experiment and for a total of 172 doses (total elapsed time of 203 days) in two additional experiments.

Neutron Doses of 17.5 n to 240 n. The purpose of this phase of the ex-

perimental work was to study the effects of a relatively wide range of neutron doses on the general condition of the rats and also on such specific changes in the blood as might occur.

For the higher neutron doses, four groups of six male rats each were irradiated with one to four 60-n doses on consecutive days for accumulative doses of 60 n, 120 n, 180 n and 240 n, respectively. Four other groups of

TABLE 1

Radiation Data for All Experiments

Irradiation Data				Total Time of Observation	Animal Data		Box Number†
Total Neutron Dose	Daily Dose	Time per Day	Total Days of Irradiation*		No. of Rats	Sex	
n	*n*	*min.*		*day*			
0	—	—	—	36	36	male	—
17.5	17.5	58	1	36	6	male	4
32.5	32.5	108	1	36	6	male	4
47.5	47.5	158	1	39	6	male	4
62.5	62.5	208	1	39	6	male	4
60	60	200	1	33	6	male	4
120	60	200	2	24	6	male	4
180	60	200	3	7	6	male	4
240	60	200	4	8	6	male	4
120	10	33	12	60	6	male	4
				244	5	female	4
0	—	—	—	247	4	male	—
0	—	—	—	248	4	female	—
310	1.8	50	172	247	6	male	6
310	1.8	50	172	248	6	female	6
452	1.8	50	251	330	12	female	6

* All irradiation was carried out on consecutive days with the exception that those receiving twelve 10-n doses were irradiated in 14 days with no irradiation on the third and fourth day, and those rats receiving repeated 1.8 n were irradiated daily except Sundays.

† The box number refers to the corresponding data given by Enns *et al.* (7) for the procedure of irradiation of experimental animals.

six male rats each were irradiated on a single day with doses of 17.5 n, 32.5 n, 47.5 n and 62.5 n, respectively. Observations on these animals were limited primarily to gross pathology, and to changes in body weight and total leukocyte counts. No histological study was made of any tissues from the rats in this series of doses. Blood hemoglobin determinations by the acid-hematin method were made on the rats receiving 17.5 n to 62.5 n. However, since these values showed no significant differences between irradiated and non-irradiated animals, these data are not reported in detail. In addition, when it was noted in the 60-n and 120-n

groups, that the blood hemoglobin level was decreasing in those animals surviving for more than one week, some blood hemoglobin determinations were made. From these data it was found that the animals dying after the first week showed a progressive decrease in blood hemoglobin to below 7 g. per cent. just prior to death. The important data, involving only changes in body weight and total leukocyte counts, have been graphically summarized in Fig. 1.

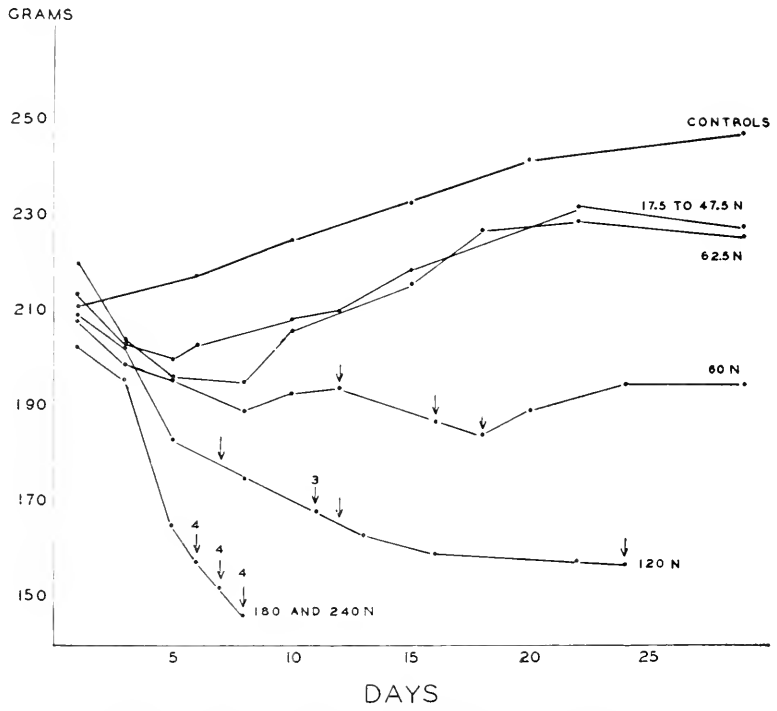


FIG. 1a. Changes in body weight from the day of the initial neutron dose for rats receiving 17.5 n to 240 n. Deaths during the course of the experiment are indicated by arrows.

At neutron doses of 17.5 n to 47.5 n, some loss in weight occurred, but since it was practically the same at all three levels of radiation, these data were averaged before being plotted in Fig. 1. At no time did any of the rats at this level of radiation show any signs of being ill. On autopsy, when the experiment was terminated, some atrophy of the thymus and testes was the only significant gross finding.

Two groups of rats receiving practically identical doses of 60 n and 62.5 n, respectively, showed quite different results. Three of the six rats receiving

60 n died in 12 to 18 days, lost weight continuously after irradiation, developed a moderate diarrhea, ate not at all during the first week and only sparingly thereafter, and at autopsy gave a typical picture of severe emaciation. Furthermore, in those animals that died, the leukocyte count was increasing while the erythrocyte count as indicated by blood hemoglobin

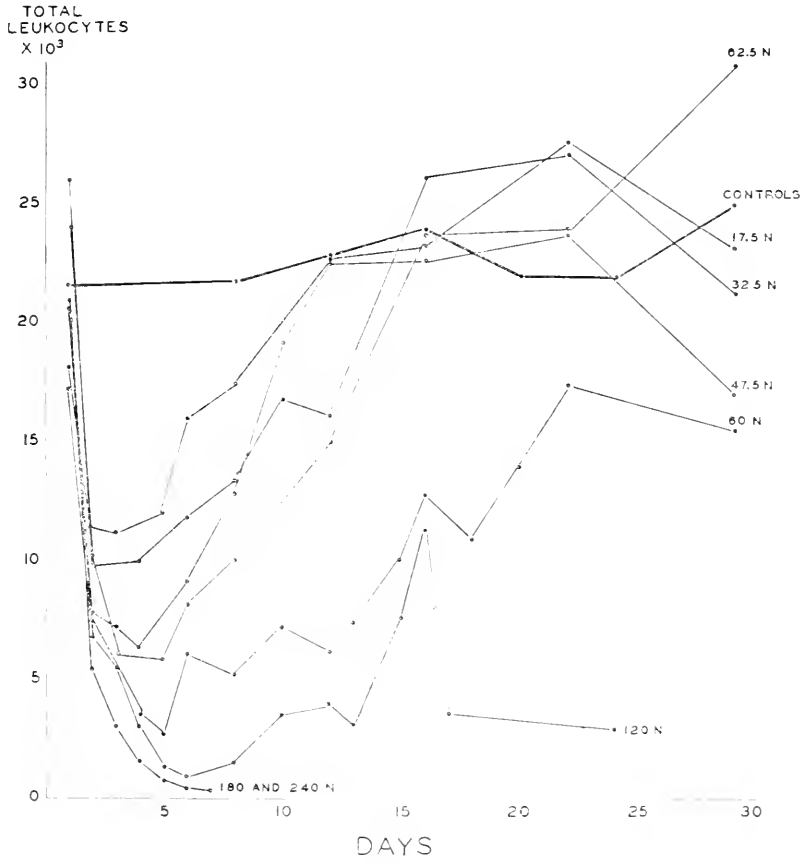


FIG. 1b. Changes in the total leukocyte count from the day of the initial neutron dose for rats receiving 17.5 n to 240 n.

determinations decreased as death approached. The survivors of the 60-n group showed a less severe reaction and ate fairly normally during the last two weeks. Some thymus and testicular atrophy was seen on autopsy. On the other hand, those rats receiving 62.5 n reacted like those at the lower levels of irradiation in that the loss of weight was less and the leuko-

cyte count did not reach as low values (see Fig. 1). In addition, these animals did not appear ill during the period of observation and were grossly normal at autopsy except for some atrophy of thymus and testes.

After a dose of 120 n of neutrons, all of the six male rats died in from 7 to 24 days. The observations made on these animals were practically the same as those described above for the 3 deaths in the 60-n group.

In the 180-n and 240-n groups, all animals died within 6 to 8 days. These rats showed a continuous and severe loss in weight, complete loss of appetite after the first 60-n dose, and a leukocyte count which approached zero the day prior to death. Furthermore, there was an acute diarrhea and on autopsy the primary finding was that of severe emaciation. In addition, the stomach was usually found to contain considerable undigested or partially digested food while the intestinal tract was devoid of any traces of food. Considerable yellowish mucoid material, frequently blood-stained, was present in the intestinal tract.

Neutron Dose of 10 n Repeated Twelve Times. In this group, 6 male and 5 female rats were irradiated at the same time and received 10-n doses of neutrons during the first fourteen days of the experiment with no irradiation on the third and fourth days. The observed mortalities, changes in weight, total leukocyte counts, differential leukocyte counts, erythrocyte counts and blood hemoglobin levels were averaged for all eleven animals and are graphically summarized in Fig. 2.

It should be noted that all males died in from 24 to 60 days after a continuous loss in weight until time of death when the average weight loss was 29.1 per cent of the initial weight. Prior to death the leukocyte counts, erythrocyte counts and blood hemoglobin levels had passed through a minimum, had increased, but had not yet reached the initial levels. Five of these rats died during the night and post-mortem changes were so far advanced at autopsy that significant data and tissues for histological study could not be obtained. The single remaining animal of this sex was sacrificed on the 60th day when comatose and gave a gross picture of severe emaciation with slight indications of atrophy of lymphoid tissues and of the testes.

On the other hand, only three of the five female rats of this group died on the 43rd, 156th, and 174th day, respectively. These deaths occurred at a time when the weight and hematological values had at least increased above the minimal values and in the last two cases had reached or even exceeded the initial values. Post-mortem changes were so far advanced on autopsy of the two rats dying on the 43rd and 174th day that no significant pathological data could be obtained.

The rat dying on the 156th day was actually sacrificed at that time when it was comatose, showing severe emaciation and complete posterior

paralysis. This paralysis was first noticed seven days previously and was of unknown etiology, unless associated with the malignant growth found microscopically in most organs. All organs were grossly normal with the

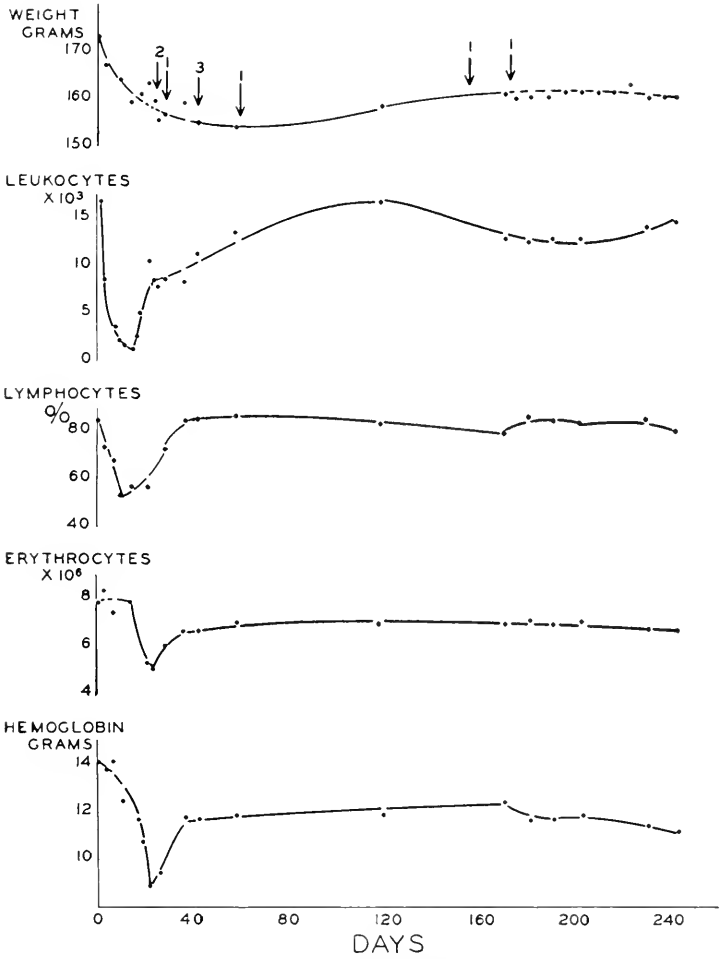


FIG. 2. Changes in body weight and hematological data from the day of the initial neutron dose for rats receiving twelve 10-n doses of neutrons. Deaths during the course of the experiment are indicated by arrows.

exception of an enlarged and dense spleen showing numerous white nodules. Microscopically, this organ was found to be massively infiltrated with highly undifferentiated tumor cells resulting in the almost complete obliter-

ation of the structure of this organ. Kidney, ovary and liver were also infiltrated by undifferentiated tumor cells although the liver showed some evidence of regeneration. The tumor seemed to have cytolytic properties since many of the liver cells appeared to be eaten away by the adjacent tumor cells.

The two female rats sacrificed on the 244th day at the termination of the experiment were grossly normal except for enlarged and hemorrhagic adrenals and hemorrhagic ovaries. Microscopically, both showed marked edema of the deeper cortical portion of the adrenal and the spleens were contracted with little blood in the sinuses. Splenic follicles were small and inactive and there were numerous neutrophils present and also small areas of hematopoiesis.

One of these rats showed a bilobular tumor, 2 x 1 x 1 cm., first noted on the 200th day, which was cut with difficulty, was capsulated and appeared granular in structure. Microscopically, this tumor was identified as an adenocarcinoma probably of mammary origin.

Since non-irradiated rats did not show any of the gross or microscopic pathologic changes described above, the changes observed in the irradiated animals probably were caused by neutrons. The available autopsy data suggest that atrophy of lymphoid tissue together with degeneration in the spleen are the primary changes produced by neutrons. The importance and significance of the presence of tumors in some of these rats will be considered later with comparable findings following repeated doses of 1.8 n of neutrons.

Neutron Doses of 1.8 n Repeated. Three different groups of rats were exposed to repeated doses of 1.8 n, as follows:

1. Twelve female rats received 1.8-n doses of neutrons, six days a week, for a total of 251 doses in 299 days (an accumulated neutron dose of 452 n) and were kept under observation for a total of 330 days.

2. Six female rats were irradiated as for the first group but for only 172 doses in 203 days (accumulated neutron dose of 310 n). These rats plus four additional non-irradiated animals were kept under observation for only 248 days.

3. Six male rats were irradiated as for the second group and together with four additional non-irradiated animals were kept under observation for only 247 days.

In these three experiments, repeated hematological checks showed that there were no significant differences between the irradiated and non-irradiated animals,—all values for total leukocyte count, differential leukocyte count, erythrocyte count and blood hemoglobin levels being within normal limits. Only the rate of growth as indicated by the changes in body weight showed any significant variation between irradiated and non-

irradiated rats so that only these data have been graphically summarized in Fig. 3.

When all of the non-irradiated rats, both female and male, were sacrificed after 247 and 248 days on termination of the experiments, no significant gross or microscopic pathologic changes were observed in any organs.

Gross Pathology

Females—251 doses of 1.8 n: The fur of these animals was in poor condition. Atrophy of the lymphoid tissue was general together with enlarged

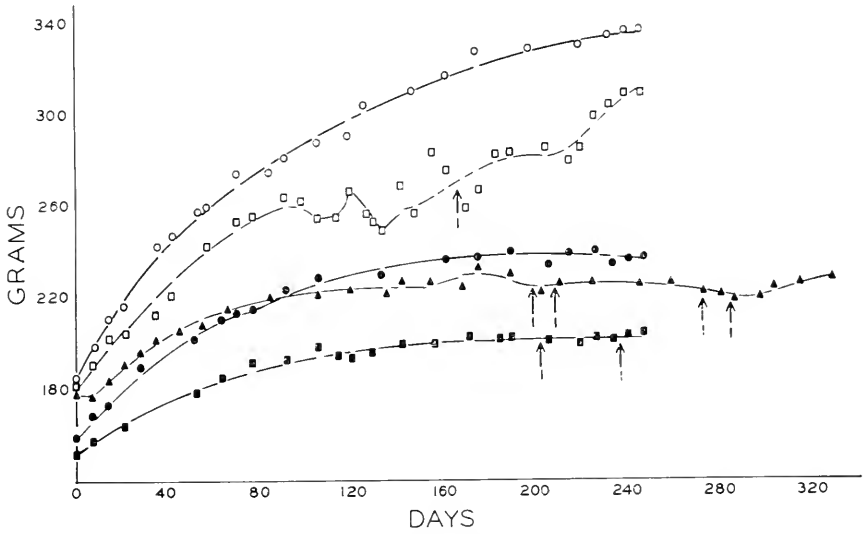


FIG. 3. Changes in body weight from the day of the initial neutron dose for rats receiving repeated doses of 1.8 n. Deaths during the course of the experiment are indicated by arrows. ○ Control males. ● Control females. □ Irradiated males receiving 172 × 1.8 n. ■ Irradiated females receiving 172 × 1.8 n. ▲ Irradiated females receiving 251 × 1.8 n.

and hemorrhagic ovaries. There were some instances of consolidation of one or both lungs to varying degrees, possibly associated with tumor metastases. Congestion and enlargement of the uterus was also frequently noted. Eight of the twelve animals showed definite tumors on gross examination as summarized in Table II.

Females—172 doses of 1.8 n: The fur of these animals was in poor condition. With the exception of a hemorrhagic condition of the ovaries all organs were grossly normal. The data for the two tumors found in one of these six females have been summarized in Table II.

Males—172 doses of 1.8 n: A poor condition of the fur and some slight

TABLE II

Data on Tumor Growths Observed Following Neutron Irradiation of Rats

Rat No.	Neutron Dose		Time Growth First Observed	Time of Final Study	Size of Growth	Site	Microscopic Characteristics
	Daily	Total					
	n	*n*	*day*	*day*	*cm.*		
IX-7	10	120	200	211	2 x 1 x 1	Dorsal surface of left shoulder	Adenocarcinoma of mammary origin
IX-8	10	120	156	156	—	Spleen, kidney, liver, ovary	Undifferentiated malignant tumor of abdominal viscera
X-2	1.8	367	225	330	2 x 2 x 2	Right axillary region	Adenocarcinoma of mammary origin
					5 x 5 x 3	Left axillary region	Chronic mastitis
X-3	1.8	367	225	285	3 x 1 x 2	Left axillary region	Papillary carcinoma of mammary origin
					Size of pea	Mediad to 1st tumor	Papillary carcinoma of mammary origin
X-4	1.8	320	207	330	4 x 3.5 x 2	Right axillary region	Both adenocarcinoma of mammary origin
					4 x 3 x 3	Post. region of left abdominal wall	
X-6	1.8	337	218	330	2.5 x 3 x 2	Left axillary region	Both adenocarcinoma of mammary origin
					3 x 4 x 2	Post. region of left abdominal wall	
					Small	Kidney	Nephroma
X-7	1.8	369	239	330	2 x 1.5 x 1	Right clavicular region	Hematoma-like (not studied microscopically)
					3 x 2.5 x 1	Right pelvic area of abdominal wall	Unidentified

X-8	1.8	367	225	330	2.5 x 2.5 x 2 3 x 2 x 1.5	Right clavicular region Right lateral area of abdominal wall	Both adenocarcinoma of mammary origin
X-10	1.8	306	198	198	5 x 6 x 3	Lower right quadrant of abdominal wall	Reticulum cell sarcoma of lymphoid origin
X-12	1.8	420	272	272	1 x 0.7 x 0.5	Left lateral abdominal wall	Unidentified
XI-B1-5	1.8	263	170	236	4 x 3 x 2.5 5 x 5 x 4	In left angle of head and shoulder Lower right quadrant of abdominal wall	Both mixed carcinoma and sarcoma

degenerative changes in the testes were the only significant gross findings observed at autopsy. None of these rats showed any gross indications of tumors during the period of observation.

Microscopic Pathology

Females—251 doses of 1.8 n: The spleen showed varying degrees of atrophy with inactivity of the germinal centers. Numerous neutrophils and foci of hematopoiesis were also present. The thymus showed atrophy with occasional edematous areas.

Females—172 doses of 1.8 n: The spleen and thymus showed various degrees of atrophy while the ovaries were frequently congested, although germinal follicles were present and oogenesis was going on.

Males—172 doses of 1.8 n: The spleen showed numerous foci of hematopoiesis and a few neutrophils and the germinal follicles were somewhat smaller than normal. The thymus in one rat showed slight atrophy of the germinal centers. All testes were markedly atrophied with little or no indications of spermatogenesis.

Under these conditions of irradiation, only moderate changes were produced outside of the action responsible for the initiation of malignant growths.

DISCUSSION

Before considering the detailed discussion and interpretation of the experimental data reported, the purposes and limitations of these data must be realized. The data, though incomplete, are presented at this time to serve as a guide to other workers in this field and as a basis for future detailed studies concerning the mechanism of action of neutron rays on organisms and biological systems. The scope of the data is definitely limited for the following reasons:

1. The data are based on a relatively small number of rats of a single strain. Whether or not comparable information would be obtained for other strains of rats as well as for other mammalian species remains to be investigated.

2. Only a small number of neutron doses has been studied, but, nevertheless, these cover what may be considered as heavy, "acute", lethal doses (120 to 240 n) and light, "chronic", doses (1.8 n daily) as well as some intermediate values (17 to 60 n in a single day). Therefore, the data give an over-all picture of the results that may be expected from whole-body irradiation of white rats.

3. Animal studies were limited in this preliminary survey to general hematology, body weight and gross pathology.

In the subsequent discussion, the above limitations must be kept in mind together with the fact that final interpretation or explanation of many

of the observed results will have to await the accumulation of additional data.

Neutron Doses of 17.5 n to 240 n. The primary purpose of this phase of the experimental work was to determine the general relationship between the general effects on rats and the specific effects on the blood as produced by varying doses of neutrons.

The results indicate that with neutron doses of 180 n and 240 n death occurs in from 6 to 8 days with little or no difference between the two doses. These acute deaths are characterized by a continuous loss in weight and extreme leukopenia. The changes in body weight are very striking and death occurred at a point when the loss in weight was equivalent to 20 to 30 per cent of the initial weight. The rats were not observed to eat any food after the first dose of neutrons. A weight loss for any one rat exceeding 15 per cent of the initial weight together with complete loss of appetite and acute diarrhea followed by a mucous discharge may be considered as definite signs of an irreversible reaction which will terminate in death.

Since these animals showed a general appearance of acute starvation, a group of rats was deprived of food so that a picture of starvation could be obtained for comparison with that produced by irradiation. In these starved animals, a weight loss of 35.4 per cent was observed at the end of 10 days with the death of only one of twelve rats. The survivors when again given access to food regained their initial weight within two weeks. The rate of loss of weight was practically identical for both the starved, non-irradiated animals and the irradiated animals (180 n and 240 n). In fact, the only observable difference between these two groups was that only one of the starved animals died in 10 days while all of the irradiated ones were dead in 7 to 8 days. Apparently in irradiated animals some mechanism of the digestive system is disturbed if not completely inhibited. From autopsy evidence, a pyloric spasm may have occurred inhibiting the passage of food from the stomach to the duodenum. In addition, some action on the gastric enzymes may be assumed on the basis of the partially digested condition of the food found in the stomach at autopsy. At high neutron doses, the pyloric spasm might be considered as complete and irreversible and death as being due, at least in some degree, to starvation and the resulting tissue destruction. These data support the conclusion of Lawrence and Tennant (5) that tissue destruction plays an important part in the mechanism of acute action of neutrons. It would be interesting to see if such animals could be saved or their life prolonged either by forced feeding with duodenal tube or by intravenous feeding with predigested proteins. Such experiments might show that other effects of neutrons have been produced which are masked by the rapid and fatal progress of starvation.

Neutron doses between 60 n and 120 n are apparently in the critical range since from the higher dose and upward all rats died, while at the 60 n and 62.5 n level only three of twelve died. Deaths at this level of irradiation were comparable to those at the higher level in that there was a continuous weight loss until death. However, at the lower irradiation level the leukocyte count was increasing instead of being at a minimum. Furthermore, when the animals survived for more than ten days, deaths were always preceded by a gradual decrease in the blood hemoglobin levels. These results suggest that the leukopoietic system is regenerating prior to death and that the erythropoietic system has been damaged. Additional data are required before any definite conclusion can be drawn concerning the relation between the changes in the leukopoietic and erythropoietic systems and death by neutron rays.

That three of six rats died following a 60-n dose and none following one of 62.5 n can be accounted for on the basis of variation in the initial weight of the rats. Those dying after 60 n had an initial average weight of 194 g. while the survivors averaged 221 g. This latter value is practically identical with the average initial weight of 220 g. for those rats receiving 62.5 n and the over-all reactions of these nine animals were the same. These data indicate that the lower the body weight, the more sensitive rats are to neutrons. The one rat at the 120-n level that died in 7 days further supports this relationship since its initial weight was 154 g. as compared to 220 g. for the other five animals. This general relationship was expected since it is well known from the fields of radiology and toxicology that the sensitivity to foreign stimuli, whether physical or chemical, increases with decreasing body weight.

At neutron doses below 60 n, the animals showed a slight decrease in weight and also in the total leukocyte counts, both characteristics decreasing to a greater extent the higher the neutron dose. Apparently the leukopoietic system is specifically, but not irreversibly, injured together with general tissue destruction at low levels of neutron doses.

Neutron Dose of 10 n Repeated Twelve Times. In this group, in which six male and five female rats received twelve 10-n doses of neutrons during the first 14 days of the experiments, initial severe leukopenia followed by erythropenia and decrease in the blood hemoglobin levels were observed for all animals. In addition, the values for these three characteristics showed a tendency in all animals to return to normal levels. However, the males showed a continuous loss in weight until all animals had died by the end of 60 days while in the females, after a slight initial loss in weight, a fairly normal growth rate was maintained. Furthermore, only three of the five female rats died during the 244-day observation period. From this experiment it would appear as if the males were much more

sensitive to neutron irradiation than the females. However, since only eleven rats were involved in this experiment and none of the other experiments showed a similar variation in sensitivity dependent upon sex, additional experimental data under comparable conditions of irradiation are necessary before the significance of these results can be determined. It should be emphasized that nothing was observed during the course of this experiment that might account for the apparent sex difference.

Of primary significance in this experiment was the appearance of a tumor in one of the female rats (see rat IX-7, Table II), since this was the first indication of the initiation of tumor growth by neutrons. Microscopically, it was found that one other animal in this experiment (rat IX-8, Table II) showed evidence of extensive malignant growth in the abdominal organs. This latter case was the only instance of tumor formation in which gross evidence thereof was not apparent and also in which the abdominal organs were involved. The initiation of tumors by neutron irradiation will be discussed further in connection with the data for those animals receiving repeated irradiation with 1.8 n of neutrons.

Repeated Doses of 1.8 n. In this group there were three different experiments in which rats received 1.8 n of neutrons, six days a week, for several months. Of greatest significance is the fact that although there were no changes in the peripheral blood, neutron irradiation did produce a definite reduction in the rate of growth and furthermore numerous tumors developed in the irradiated rats. The change in the growth rate was only apparent on comparison with the corresponding non-irradiated rats. The rats throughout the experiments seemed to be in quite good condition although some roughness of the fur was apparent. This growth reaction to repeated low doses of neutrons is of little value as a criterion of injury when applied to the safety of radiologists since comparative data would not be available.

Since no previous report on the initiation of spontaneous tumors by neutrons has been found in the literature, further discussion of this phase of the results is desirable. First it should be noted that no spontaneous tumor formation has been observed in the Brooklyn Strain of rats as maintained in this laboratory so that these results must be considered as either direct or indirect action of neutron irradiation. Tumor formation was first observed following twelve 10-n doses where it was preceded by definite and severe hematological changes indicative of neutron injury. However, after repeated doses of 1.8 n, there was no definite indication of neutron injury prior to the appearance of small subcutaneous tumors. These tumors were palpable from 170 to 239 days from the start of irradiation after from 146 to 205 doses of 1.8 n (accumulative doses of 263 n to 369 n). However, following twelve 10-n doses, the rats developed tumors after an

accumulated dose of 120 n given in only twelve doses. Additional experimental data are required before the minimal dose of neutrons and the conditions of irradiation necessary to induce spontaneous tumors can be determined.

All of the tumors observed from the gross study were found between the skin and muscle layers with no involvement of the latter tissue. From Table II it can be seen that there was a tendency for two tumors to appear together, usually in the axillary region with some in the lateral abdominal wall and inguinal regions. Tumors were identified histologically as predominantly arising from mammary tissue with the formation of chronic mastitis and adenocarcinoma. One rat (X-3, Table II) showed papillary carcinoma of mammary origin, a second (X-10) a reticulum cell sarcoma of lymphoid origin while a third (XI-B1-5) developed a mixed carcinoma and sarcoma. No tumors were found in the thoracic or abdominal organs although some evidence suggested metastases into some of the internal organs.

These findings of spontaneous tumor formation during or after neutron irradiation are of prime importance in regard to the safety of radiologists since exposure to low neutron doses over long periods of time may cause serious injury without any prior warning from hematological changes in the peripheral blood. For this reason it seems vitally important that this phase of the neutron problem be investigated further and additional data be obtained on the relationship between repeated exposures to low doses of neutrons and spontaneous tumor formation.

The present data will not permit of extended comparison of the formation of tumors by neutron rays with those caused by ultraviolet light (Blum, 8), by mesothorium (Gricouroff, 9), and by X-rays (Furth *et al.*, 10, 11). However, it should be noted that Furth *et al.* reported the presence of ovarian tumors after X-rays whereas no tumors of this organ were observed after neutron irradiation. Whether or not this indicates a difference between the action of X-rays and neutrons cannot be concluded at this time.

The data herein reported indicate that neutrons cause effects comparable to those of X-rays, particularly with respect to changes in the peripheral blood and in the body weight of irradiated animals. In addition, both types of radiation which can produce spontaneous tumors have been used for the treatment of cancer. Recently, Gilman and Philips (12) and also Rhoads (13) have reported that β-chloroethyl amines produce leukopenia and also have been used in the treatment of cancer. These compounds apparently have an action quite comparable to that of X-rays and neutrons. It would, therefore, be interesting to see whether these amines would also induce spontaneous tumors if given in extremely low doses over

a long period of time. If such a similarity in action could be demonstrated, it would make available another method for the induction and study of such tumors in experimental animals.

SUMMARY

1. Neutron doses of 180 n and above caused death in from 6 to 8 days accompanied by extreme loss in weight indicative of tissue destruction and also a maximum leukopenia.

2. The median lethal dose for deaths occurring within two weeks lies between the 60-n and 120-n levels. In this range, death was associated with a severe weight loss but with recovery from the extreme leukopenia as indicated by increasing leukocyte counts prior to death. In addition, erythropenia was present for the week prior to death.

3. Neutron doses from 17.5 n to 47.5 n caused some leukopenia and weight loss followed by complete recovery. At this level of irradiation, the rats did not appear to be seriously ill at any time during the month's period of observation.

4. A reduced rate of growth and the formation of tumors but without changes in the hematological picture of the peripheral blood was found following neutron doses of 1.8 n given six days a week for seven and nine months, respectively.

5. Of 28 rats surviving for more than 150 days after repeated doses of neutrons, 11 showed malignant tumors, which were classified as follows: mammary adenocarcinoma 6, mammary carcino-sarcoma 1, nephroma 1, reticulum cell sarcoma 1, unidentified 2.

REFERENCES

(1) Stone, R. S., in "Medical Physics", edited by Otto Glasser, The Year Book Publishers, Inc., Chicago (1944), pp. 812–16.
(2) Lawrence, J. H., *Am. J. Roentgenol. Radium Therapy*, **48**, 283 (1942).
(3) Henshaw, P. S., *J. Natl. Cancer Inst.*, **4**, 477, 485, 503, 513 (1944); **5**, 233 (1945).
(4) Lawrence, J. H., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 124 (1936).
(5) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
(6) Yamashita, H., *Gann* **31**, 629, German Abstract, 651 (1937); *Nature*, **141**, 416 (1938).
(7) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(8) Blum, H. F., *J. Natl. Cancer Inst.*, **4**, 75 (1943).
(9) Gricouroff, G., *Compt. rend. soc. biol.*, **139**, 558 (1945).
(10) Furth, J., and Furth, O. B., *Am. J. Cancer*, **28**, 54 (1936).
(11) Furth, J., and Butterworth, J. S., *Am. J. Cancer*, **28**, 66 (1936).
(12) Gilman, A., and Philips, F. S., *Science*, **103**, 409 (1946).
(13) Rhoads, C. P., *J. Am. Med. Assoc.*, **131**, 656 (1946).

Chapter 5

A STUDY OF POSSIBLE REACTIONS OF MICROORGANISMS TO SUBLETHAL BOMBARDMENT WITH NEUTRONS

By ROBERT K. JENNINGS and JAMES M. GARNER, Jr.

Bacteria and other microorganisms are far from simple in their structure and metabolism. Nevertheless, they would appear to offer a certain amount of freedom from confusing side effects, when the fundamental action of neutrons on living matter is under consideration. When multicellular organisms are subjected to neutron bombardment, secondary reactions to altered balance among organs, groups of cells, or even enzyme systems are likely to be encountered, and it may be very difficult to decide whether a given phenomenon should be attributed to the bombardment directly, or to injuries produced in another part of the body.

It was known (1) that sufficient dosages of neutron bombardment would exert a bactericidal influence on vegetative cells of *Escherichia coli* and on the spores of *Bacillus mesentericus*. The intensity of the irradiation required to achieve these results, however, was over 1000 n units of fast neutrons. Unfortunately, it was necessary, during the period of these observations, to give priority to pressing practical problems, which limited the availability of the cyclotron. For the most part, it was not feasible to subject our cultures to more than about a fifth of the required dosage. Accordingly, we have been limited to the consideration of possible effects of sublethal exposures.

As might be anticipated, the results obtained were largely negative, but may be of some value in guiding the efforts of future investigators.

MATERIALS AND METHODS

Test Organisms. *Escherichia coli* were used in the majority of the experiments, since they have little tendency to form clumps or chains, are sufficiently motile to form aliquot samples readily, are easy to handle, and because they had already been used by other investigators in studies on the effects of various types of irradiation. Preliminary tests, moreover, seemed to indicate that a mitogenetic response, reminiscent of the growth stimulation which Hollaender and Claus (2) found with sublethal exposure to ultraviolet light, might result from exposure to relatively small intensities of neutron bombardment.

Euglena were also considered as possible test organisms. They were found to be ill suited, however, primarily because they appeared to be

enormously resistant. In one instance, 5000 n units were applied to a young culture of euglena without diminishing the number of active individuals to be found in a microscopic field, and without giving rise to a greater number of inactive or distorted specimens. It is true, however, that in this sample as well as in others exposed to 2500 n and 1250 n units respectively, there were macroscopic changes which became apparent on standing 48 hours. In unirradiated controls, free-swimming organisms were seen as a fairly uniform haze in the liquid, while in the bombarded samples a water-clear zone roughly proportional to the intensity of bombardment appeared at the top of the medium, and a dark green layer of sediment collected on the bottom of the tube. Repeated microscopic examination still failed to establish any visible differences between exposed and unexposed samples.

The difficulty of obtaining representative samples from euglena cultures is considerable, owing to their tendency to congregate in corners, such as the angle formed by a meniscus and the wall of a small vessel, and to various tropisms. The same factors render even direct counting methods extremely inaccurate. Such considerations led us to abandon attempts to decide whether the phenomenon observed represented actual killing of the cells or perhaps a sort of auto-agglutination due to a change in acidity or tonicity of the medium in which the cells were bombarded.

Culture Media. Bacteria were grown in beef heart infusion broth (Difco), usually in 250 cc. Erlenmeyer flasks containing 100 cc. of medium. Plate counts were made by placing measured volumes of appropriate dilutions in sterile Petri dishes, then pouring in and thoroughly mixing a thin layer of the clear potato medium used by Hollaender and Claus (2). Plates and primary cultures were incubated at 37°C. When frequent counts were to be made on a subculture, however, the medium was maintained at room temperature both before and after planting in order to avoid frequent changes resulting from opening and closing the door of an incubator.

Occasionally, experiments were made with cultures which were considerably diluted in sterile saline before bombardment, in order to eliminate errors which might arise as a result of the effect of the neutrons on the nutrient medium rather than the cells themselves. Such dilutions, as well as those required in making counts, were made in sterile saline which was measured aseptically into sterile vessels immediately before use.

Neutrons. It was known at the outset that the available intensity of neutron bombardment was likely to prove inadequate to bring about readily observed responses in bacteria. We wished, therefore, to make maximum use of the neutrons available to us, and if possible, to increase their efficiency in some way. This might be possible, for instance, if the organisms proved to be particularly sensitive to neutrons possessed of a

certain specific amount of energy, as they are to certain specific wave lengths of light.

When neutrons pass through a sufficient layer of paraffin, their initial energy is lost as a result of many collisions with the nuclei present in the heavily hydrogenated compound. By regulating the thickness of a paraffin barrier, therefore, some control might be gained over the average amount of energy possessed by the neutrons striking the cells. A further advantage in the use of wax lay in the strong possibility that a greater number of neutrons might actually be brought to bear on the exposed cells than would have been the case if fast neutrons were used without wax. Finally, slower neutrons are more susceptible to capture by atomic nuclei, and might produce effects of an entirely different nature from those attainable with higher energy neutrons.

In order to provide some measure of the intensity of bombardment, use was made of the Victoreen 100 r condenser-type meter. The intensities were expressed as "n" units, by which it should be understood that ionization equivalent to that produced by a number of Roentgens of X-rays which would give the same reading with the meter was brought about within the meter chamber. It must be emphasized that this is not a valid estimate of the number or the energy of the neutrons involved. The neutrons do not produce any ionization themselves, but give rise to other particles which create paths of ionization in the material bombarded. Thus the amount of ionization, the value actually measured, is as much a function of the material bombarded as of the concentration and strength of the neutrons. Moreover, X-rays and gamma rays, both capable of producing ionization, are given off by the cyclotron during the production of the neutrons. The effect of the presence of these rays in the general radiation becomes apparent when "n" readings are taken with and without a barrier of lead or wax to act as a filter. Lead cases with one inch walls were used in some experiments to exclude the unwanted radiation, but the results indicated that the intensity of these rays was too slight to affect the results of our experiments. Samples described as having been bombarded in air, therefore, have been subjected to the total radiation from the cyclotron.

Wax Forms. Our first concern was to determine, if possible, the most effective amount of wax shielding. For this purpose the wax form illustrated in Fig. 1 was constructed. It consists essentially of a cylinder five inches long and ten inches in diameter, capped at either end with a hemisphere of the same diameter. From this melon-shaped figure, a section was removed as indicated, along planes which bisected one of the hemispheres and extended from the diameter thus defined to the point on the opposite end of the cylinder where a perpendicular from the middle

of the first plane would intersect the circumference. A length of copper tubing was embedded along the axis of the form, to serve as specimen holder. Samples placed at intervals along the portion of this tube within the cylinder were thus protected in one direction by a wedge of wax varying from 0 to 5 inches in thickness, while all other directions were guarded by at least 5 inches of paraffin. The chart accompanying the diagram in Figure 1 indicates readings obtained by placing the chamber of a Victoreen

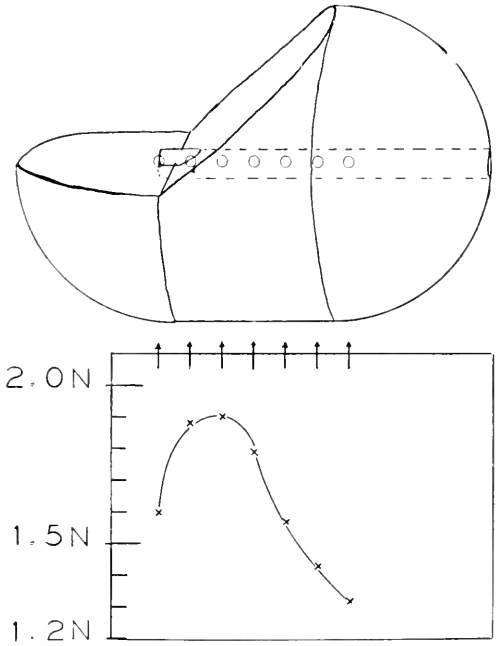


FIG. 1. *Ionization gradient in wax form.* The wedge-shaped portion is 5 inches long, and 5 inches high. The circles indicate the points (one inch apart) at which readings were taken with the 100 r meter, and the X's on the graph, the corresponding values in terms of n units per integron division. Without the wax shielding the same procedure would have given a value of 0.85 n in the same positions.

100 r meter at the points within the specimen tube indicated by circles. The values are expressed as n units per integron division (3).

It is interesting to note that readings in air—i.e., without wax shielding —in nearly the same location were invariably in the neighborhood of 0.85 n per integron division. The fact that all readings within the form were greater than 1 indicates the increase in ionization obtained from the neutrons when this large volume of wax was used.

In other experiments, smaller spherical forms were used, and the n

values were found to be less than the corresponding reading in air. This would seem to indicate that some of the radiation from the neutron source was in the form of rays which were screened out by the wax, causing an initial reduction in the amount of ionization recorded by the meter. When the wax used was sufficient to produce a large increase in the ionization attributable to the action of the neutrons, the n to integron ratio rose sharply, finally exceeding the sum of the ionizations produced by the total radiation when wax was not present.

Location of the Samples During Bombardment. As nearly as the dimensions of the wax form would permit, the experiments were all made with the organisms placed to receive a maximum of neutron intensity in a given time. The spot chosen was about twenty inches from the neutron source and only a few inches from the line of greatest intensity of high energy neutrons (3).

EXPERIMENTAL

Orientational Experiments. As a test of the efficacy of the wax in enhancing the effect of neutrons on bacteria, organisms were exposed at three points beneath the wedge-shaped section of the large paraffin shield, while controls were exposed at the same time without shielding, and a second set of controls was retained without bombardment. In one experiment, it was possible to expose the organisms to sufficient bombardment to give an average "n" reading of 1000 in the specimen tube. The irradiated controls were placed without shielding of any sort immediately against the wall of the neutron source, where they might be expected to receive a maximum bombardment with fast neutrons. In this position, it is true, they were also exposed to slow neutrons scattered from the wax form.

The samples were measured out as accurately as possible with a capillary pipette, and aspirated into individual capillary tubes which were then sealed. All samples were prepared simultaneously from the same 48-hour broth culture of *E. coli*. They were then distributed in groups of six by random choice. One group was placed beneath the thin edge of the wedge, one group in the middle and one at the thick edge within the specimen tube. The exposed control was taped to the wall of the cyclotron. Following bombardment, all capillaries were unsealed and the contents blown out onto the surface of sterile agar plates and streaked. The results are indicated in Table I.

It was extremely unfortunate that this crude experiment could not be thoroughly checked by similar runs with more exact technic. The results gave strong indication, as can be seen, that the slower neutrons, which are capable of producing ionization exceeding that of the total radiation without wax, exerted a stimulus to further reproduction of the resting culture.

The fact that the gradient effect of the wedge was not apparent, and that the exposed control, which presumably was exposed to a smaller number of rebounding slowed neutrons, showed some stimulation, would suggest that the action, if real, is a property of the very slow neutrons which probably are present in the entire length of the specimen tube in equal concentration throughout bombardment.

As it was not possible to make numerous repetitions of the experiment, we turned our attention to the elimination of this "fogging" of the gradient which must inevitably occur in a single wax form, due to the tendency of the neutrons to "wander about" from collision to collision within the paraffin before reaching points all along the specimen chamber. Paraffin spheres of various sizes were constructed, ranging in diameter up to that of the melon-

TABLE I

Response of E. coli to Heavy Bombardment Within the Gradient-Producing Paraffin Form Illustrated in Figure 1

	Unexposed Control	Exposed Control	Thin Edge of Wedge	Middle of Wedge	Thick Edge of Wedge
Largest No. of colonies	226	1330	3340	3420	2940
Smallest No. of colonies	2	47	517	833	631
Average No. of colonies	66	660	1440	1460	1410

Six samples in individual capillaries were exposed in each location. The results express the number of viable organisms found in the capillaries containing the largest number and the smallest number for each group. The average of the six is also indicated.

shaped form itself. The n per integron division values found in the center of these spheres were as follows:

Diameter of Ball	0 inches	$1\frac{3}{16}$ in.	$2\frac{5}{8}$ in.	5 in.	10 in.
n Integron Division	0.85	0.687	0.75	0.79	0.8

It will be noticed that the 10-inch ball did not give rise to ionization sufficiently intense to exceed that obtained without wax. It follows that the large volume of wax used in the melon-shaped form was required to produce neutrons with this property, and that hereafter the amount of paraffin as well as the thickness of the shield should be taken into account.

One-cc. samples of culture were exposed individually to varying amounts of neutron bombardment within these spheres, and accurately measured samples were plated out after appropriate dilution. As a rule, no significant difference between bombarded specimens and unexposed controls could be found. Occasionally, there appeared to be a certain amount of stimulation, but the observation was not a constant one.

Confirmation of the observation made by Spear (1), that sufficiently large doses of fast neutrons are lethal, was secured in an experiment during which we were able to subject organisms to 3000 n in air. A 48-hour culture was divided into three samples, one of which was unexposed, one subjected to 300 n, and the third to 3000 n bombardment. The 300 n sample was then found to contain slightly more than the 750 million organisms per cc. present in the control, while the 3000 n sample showed a reduction of the viable count to 40 million.

Studies on Growth Curves. In the preliminary experiments, observations were made only on the population of a given culture immediately after bombardment. If it were true that sublethal dosages of neutrons exert a stimulatory effect on the growth of the organisms, this should be more apparent if the organisms were permitted to multiply for some time after exposure.

In subsequent experiments, therefore, organisms were exposed to the action of the neutrons, and then planted in fresh nutrient broth. The growth of this subculture was carefully observed and compared with similar subcultures from samples which originated in the same parent culture and were subjected to identical changes of shape, volume, temperature, and so forth, but not irradiated. As a rule, three 5-cc. samples were removed from 100 cc. of parent culture, placed in sterile tubes, and kept together thereafter except for the period of actual bombardment. One was retained as control, one exposed in air and the third to the same number of integron divisions of bombardment in a wax ball.

The results of a typical experiment are displayed in Fig. 2. The samples were taken from a 48-hour broth culture of *E. coli*. One sample was exposed to 200 integron units in a five-inch wax ball, one to 200 integron units without wax, and the third was not bombarded. Following the bombardment of the second specimen, all three were diluted—1 cc. in 99 cc. of sterile saline—and 1 cc. of this dilution was transferred to 99 cc. of fresh medium. Samples withdrawn from these subcultures at intervals were appropriately diluted and plated out. The graph represents the relation between the population of viable cells and the age of the subculture.

It will be noted that the initial counts in the three samples were nearly the same, so that no great increase in population of the exposed specimens could have occurred during bombardment. The duration of the lag phase was equal for all three subcultures, and the generation time also seemed to be unaltered by the neutron bombardment. The only observable differences lay in the degree of apparent decrease during the lag phase—a phenomenon which must be interpreted cautiously at best, since agglutination would account for it as easily as death of the cells.

Experiments were undertaken to study the lag phase more carefully.

Figure 3 shows that careful technic, with counts made at fifteen minute intervals, produced results which indicated that the apparent effect on the lag period was not significant.

A repetition of the first experiment, with the initial 1/100 dilution of the culture in saline made before bombardment rather than after, was interesting in that similar results were obtained except that there was a general slow rise in count during the lag phase, rather than a decrease. The fact that the controls in both instances showed the same trend as the bom-

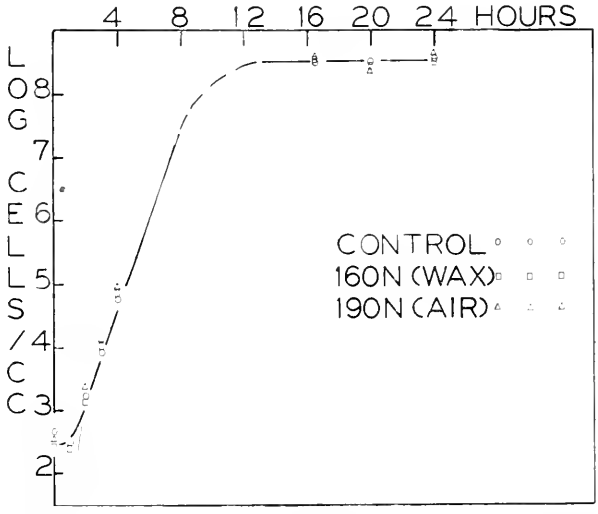


FIG. 2. Comparison of the growth curves obtained with irradiated and non-irradiated inocula of *Escherichia coli*. The line in this and in subsequent figures is an arbitrary graph to aid in assessing the accuracy of the experimental procedure, and the degree of variation of experimental values from the theoretical normal. The dotted portion of the line represents the probable development during a period at which no samples were taken.

barded samples proved that the behavior during the lag phase was not due to the neutron bombardment. It appears that holding the organisms in saline at room temperature for a few hours before planting affects them in such a way that they start to grow slowly almost at once, while transfer directly from the resting culture to fresh medium, even though the cells are passed through saline momentarily on the way, may result in the death or "clumping" of a certain portion of the seeding.

It has often been found that actively dividing cells are more vulnerable to the action of various forms of irradiation than similar resting cells. In a few experiments, such as that illustrated by Fig. 4, the parent culture was

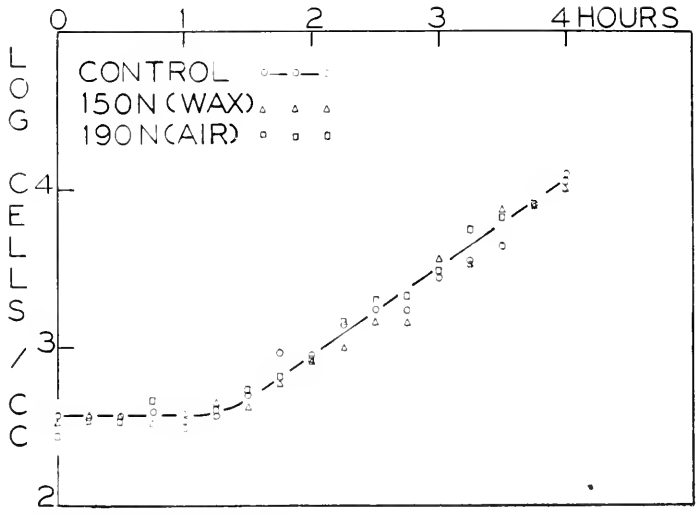


FIG. 3. Comparison of the development, during the first four hours after planting, of cultures obtained from irradiated and unirradiated samples of *E. coli*. Counts made at 15 min. intervals. Subculture maintained at 28°C.

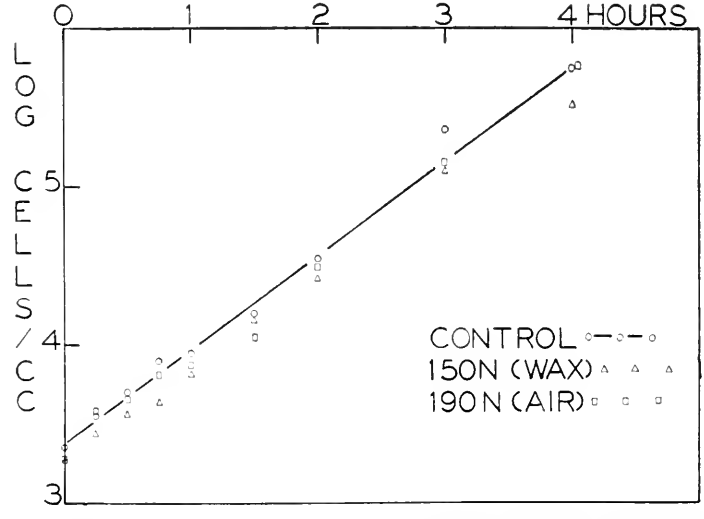


FIG. 4. The development of cultures from samples irradiated during active multiplication, compared with similar unirradiated sample.

planted only a few hours before exposure. The organisms were thus in the logarithmic phase of development during bombardment. They remained so when transferred to fresh medium, so that no lag phase was found.

The graph shows that no significant difference in either population or rate of growth could be observed following exposure to 200 integron divisions of neutron bombardment.

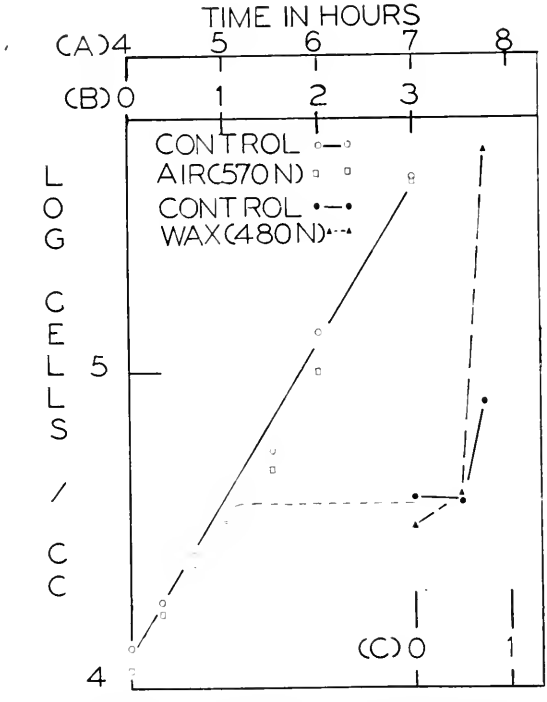


FIG. 5. Growth of organisms irradiated during early part of resting phase.

(A) Age in hours of culture from which irradiated and control samples were taken

(B) Age in hours of subculture planted with organisms exposed to 570 n without wax shielding

(C) Age in hours of subculture planted with organisms exposed to 480 n in 10 in. wax ball.

The light dotted line indicates the probable course of development of the original culture in the small sample tubes prior to exposure. In this experiment, organisms exposed without wax were irradiated during active multiplication, while the organisms exposed in wax had reached full development before bombardment.

In experiments such as the one illustrated it was necessary to bombard the two samples separately, since the slow neutrons produced in the wax would find their way to the "air" sample to some extent, and reduce its value as a control. This meant that it was necessary to hold one sample for some little time after bombardment before transplanting, in order to plant all three samples simultaneously.

Fig. 5 represents an experiment in which we sought to overcome this

difficulty by making use of two control samples, and planting immediately after bombardment in each case. Such a procedure might possibly show up reactions which occurred during bombardment and were subsequently masked by overgrowth of the unaffected cells before the transplant was made.

In the experiment illustrated, trouble with the cyclotron upset the proposed time schedule. Instead of being about three hours old when irradiated, the "wax" sample was actually six hours old. While the initial 100 cc. culture would still have been multiplying actively at this time, it appeared that the small sample—possibly due to difference in surface-depth ratio—had passed into the resting phase at a much lower level than would be anticipated. It is interesting to note that the sample exposed first grew logarithmically in the transplant from the very beginning, while the second sample and its control required a lag period. The fact that the lag period in both bombarded sample and control were of about the same duration argues against the reality of the apparent stimulation of growth under these conditions. The behavior during logarithmic growth seems to indicate a shorter generation time for the sample bombarded during the early part of the resting phase, but it must be borne in mind that much higher counts were anticipated and dilutions made accordingly. The counts, therefore, must be considered inaccurate.

In general, it was considered to be fairly well demonstrated that neither death nor stimulation resulted from exposure of *E. coli* to doses of neutrons considerably less than those employed by Spear (1); indication of stimulation with very slow neutrons, however, remains to be confirmed.

DISCUSSION

Neutron bombardment might be expected to kill organisms outright, as indicated by the work of Spear (1), or the neutrons might possibly alter the metabolism or morphology of the organisms in some way, as has been shown to occur in the case of molds (4). In our experiments, the first type of reaction would have been observed as a reduction in the initial viable counts in the subcultures from bombarded samples. Such a decrease was not found, and it is further noted that the actual number of viable organisms in the seedings were such as would have been present in similar amounts of culture taken from these same subcultures at the conclusion of the growth period. That is to say, the samples appeared to contain as many viable organisms as the medium was capable of supporting.

The failure of neutron bombardment, in intensities less than 1000 n, to alter the rate of growth or duration of the lag period constitutes strong presumptive evidence that no profound alteration in the metabolism of the

organisms resulted from their exposure to sublethal doses of neutrons. It is possible, of course, that certain individual cells were affected, and the fact was hidden by the subsequent development of large numbers of unirradiated organisms. However, no abnormal colonies were found on plates made at the beginning of the development of subcultures, even when several such plates were made before active growth began.

On the other hand, changes in the total population of resting cultures exposed to very low energy neutrons seem to indicate that there may be a specific sensitivity to these particles. Such a hypothesis might account for the occasional stimulation found in even the more exact experiments, since the radiation from the cyclotron contained neutrons of this type in low concentration. If it is assumed that the organisms are extremely sensitive to slow neutrons, it might be possible to account for the erratic finding of stimulation on the grounds of varying concentrations of slow neutrons in the total radiation at different times.

Such an assumption has not been proved by these experiments. More work, with larger volumes of wax, is required before conclusions can be drawn. It appears certain, however, that fast neutrons, delivered in intensities much less than that represented by the designation 1000 n in our experiments, do not produce any observable effect on the over-all behavior of the microorganisms studied. If such neutrons are slowed sufficiently to bring the "n" reading produced by the neutrons to a higher level than that produced by the total radiation from the cyclotron without the presence of wax, growth may possibly be stimulated.

CONCLUSIONS

E. coli and euglena are relatively resistant to neutron bombardment. Confirmation of the observation that neutron intensities over 1000 n will produce demonstrable bactericidal effects on *E. coli* was obtained; such dosages were also found to affect euglena cultures in some way which restricts their motility. Smaller dosages of high energy neutrons have little or no observable effect on the culture as a whole. There is some indication, which merits further study, that *E. coli* may have a specific sensitivity to very low energy neutrons.

REFERENCES

(1) Spear, F. G., *Brit. J. Radiol.*, **17**, 348 (1944).
(2) Hollaender, A., and Claus, W. D., *Natl. Research Council Bull.* No. 100 (1937).
(3) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(4) Myers, W. G., and Hanson, H. J., *Science*, **101**, 357 (1945).

Chapter 6

EFFECT OF NEUTRONS ON THE RESISTANCE OF MICE TO STAPHYLOCOCCUS INFECTION

By J. O. ELY and M. H. ROSS

Neutron irradiation of animals was found by Lawrence *et al.* (1, 2) to reduce the white blood cell count and to have a profound effect on the lymphoid tissues. Lawrence and Tennant (3) concluded that, after irradiation with doses of X-rays or neutrons sufficiently large to result in the death of mice within a few days, infection was not necessarily a finding. When the doses were decreased, however, and the animals lived longer, bacteremia was usually observed. Resistance to infection therefore appears to be reduced in animals exposed to neutron radiation.

Three groups of 30 mice, of the Detweiler strain, averaging nearly 20

TABLE I

Mortality Frequency among Mice Injected with Bacteria

Group	No. of Mice	Injected with Bacteria	Successive 24-Hour Periods							
			1st	2nd	3rd	4th	5th	6th	7th	Total
Irradiated	30	yes	1	3	19	1	3	0	3	30
Non-irradiated	30	yes	0	0	1	0	0	0	0	1
Irradiated	30	no	0	0	0	0	0	0	0	0

grams, were allowed water and food *ad lib.* Two of these groups were irradiated with 84.6 n in Box No. 7 (Enns *et al.* (4)).

The organisms of a strain of hemolytic *Staphylococcus aureus*, grown on Difco heart infusion broth (agar slants), were removed 18 to 20 hours after seeding by washing with 0.85 per cent NaCl solution. This suspension of organisms was injected intraperitoneally, one injection approximately 20 hours after completion of irradiation and another 24 hours after the first injection. The amount of bacteria injected was the same for all mice.

Group I was irradiated and injected, Group II was not irradiated but was injected, and Group III was irradiated but was not injected. The frequency of deaths in the three groups during seven 24-hour periods following the time of infection is shown in Table I.

A marked decrease in the resistance of the mice to staphylococcus infection followed irradiation. This is similar to results with X-rays found

by other workers. According to Chrom (5), animals given large doses of X-rays showed increased susceptibility to bacterial infections. Schwienhorst, as reviewed by Warren and Dunlap (6), studied rats treated with X-rays; he observed microscopically that, after heavy X-irradiation, the reticulo-endothelial cells were injured and that phagocytosis by cells lining the blood channels was decreased. Knott and Watt (7) found that the leukocytes of normal and leukemic blood, irradiated with X-rays *in vivo* or *in vitro*, showed a loss of their ability to phagocytose staphylococci. It seems probable that the effects of neutron radiation in reducing resistance to infections are similar to those produced by X-radiation.

SUMMARY

Resistance of mice to infection with hemolytic *Staphylococcus aureus* was found to be reduced after exposure to 84.6 n.

REFERENCES

(1) Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 543 (1936).
(2) Lawrence, J. H., in "Handbook of Physical Therapy", American Medical Association, Chicago (1938).
(3) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
(4) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(5) Chrom, Sv. A., *Acta Radiol.*, **16**, 641 (1935).
(6) Warren, S., and Dunlap, C. E., *Arch. Path.*, **34**, 562 (1942).
(7) Knott, F. A., and Watt, W. L., *Brit. Med. J.*, **1**, 542 (1929).

CHAPTER 7

EFFECTS OF NEUTRONS ON EARLY ROOT DEVELOPMENT OF ZEA MAYS

BY MARY A. RUSSELL AND JAMES M. GARNER, JR.

Most of the work describing the effect of neutrons on plant material compares these effects with those produced by X-rays or other forms of radiation. Marshak (1) and Thoday (2) discussed the comparative effect on chromosomes, Zirkle *et al.* (3) dealt with the growth of wheat seedlings and fern spores, and Gray *et al.* (4) determined the doses of four different types of radiation which killed young bean plants. The following investigations were made to determine the sensitivity of various parts of the root system of corn (Zea mays) to neutrons.

The corn seed was "Patriot Hybrid No. 52" furnished by the Seaboard Seed Co. Although preliminary experiments showed that the seed was much more sensitive to neutrons when it was germinated than when dry (as is the case with X-rays (5)), the seed was radiated while dry in order that large doses might be built up by exposure to neutrons over a period of several days. During radiation the seed was held by glass test tubes inserted in lead cylinders. Each tube had a capacity of about twenty-five kernels. After radiation, control and treated seed was soaked for thirty minutes in a 0.05 per cent aqueous solution of mercurophen to prevent the growth of mold, then placed between layers of damp paper towelling in a large moist chamber. After about forty-eight hours in an incubator at 30°C. the primary roots were measured. The seedlings were then placed in jars of moist sphagnum moss with the roots arranged around the edge so that they could be observed through the glass. These jars were kept in the dark except when they were removed from the incubator for observation of the seedlings at twelve to twenty-four-hour intervals.

Fast neutrons (6) were used in all experiments unless otherwise specified. The fast neutrons, with an average energy of about 6 MEV, produced by the cyclotron, were utilized by placing the corn in their path, as shown by position 8 (6, Fig. 1). Lead cylinders, with walls one inch thick and with one-inch lead plugs in the ends, were used as gamma ray filters. Intensity measurements were made with Victoreen r meters in the cylinders as described by Enns *et al.* (6).

In order to obtain information concerning the intensity of gamma radiation within the cylinders, additional measurements were made with two inches of lead between the cylinders and the beryllium target of the cyclo-

tron. The readings of the r meter were slightly lower when the additional two inches of lead were used, indicating the presence of some gamma radiation even when the cylinders were used. To determine how much consideration should be given to the possible effects on dry corn of the small amount of gamma radiation present in the lead cylinders, a sample of corn was irradiated with 92-Kv. X-rays for a total dosage of 6000 r. Following germination no measurable effect was observed. Since X-rays are similar to gamma rays, and the gamma radiation received by the corn in these experiments probably would not exceed 6000 r, any possible effect of gamma rays was not considered.

Lower energy neutrons were obtained by putting the seed in lead box 7 (6, Fig. 1). Neutrons reaching this position had an average energy of about 2 or 3 MEV, as explained by Enns *et al.* (6).

Effect on Primary Roots. When low doses of neutrons were used (53–160 n), there was a slight indication of possible stimulation based on the comparison between the length of the primary roots and those of the controls. Growth of the primary root was retarded by doses of 200 n and over (Fig. 1). When 2000 n were used, the seedlings died after about 120 hours of slow growth, after doses above 2000 n they died within 72 hours, with less growth of the root. Circumstances prevented our using doses of over 80,000 n, but it is hoped that at a future time the dose of neutrons required to prevent germination may be determined. The fact that doses between 5500 and 80,000 n resulted in delayed killing after a definite and similar amount of growth suggests an effect comparable to results obtained by Collins and Maxwell (7) when they X-rayed dry corn. They found that "from 60,000 to 100,000 r units the percentage of germination remained unimpaired but all the plants died in the seedling stage", and from their other experiments discovered that doses of approximately 2,000,000 r completely prevented germination.

Effects on Adventitious Roots. While differences in the response of the primary root to various amounts of radiation seem to be the most obvious and the easiest to measure, the response of other parts of the root system have real significance. The normal corn seedling raised under the conditions of these experiments produced four adventitious roots at the first node of the shoot. The average length of each of these roots was about 40 mm. when measured 100 hours after germination started. The adventitious roots of seedlings which had received 1000 n or less showed no differences as compared with the controls, but doses of 2000 n and over produced damaging effects as shown in Table I. Smith and Kersten (8) report that after dry corn received a dose of soft X-rays sufficient to kill the seedlings after their primary roots had reached a length of 30 mm., no adventitious roots were present. A dose of 80,000 n still permitted the appearance of

adventitious roots on 17 per cent of the seedlings (Table I). In this case the primary root had reached a length of only 19 mm. before death, as compared with 30 mm. in the X-rayed plants.

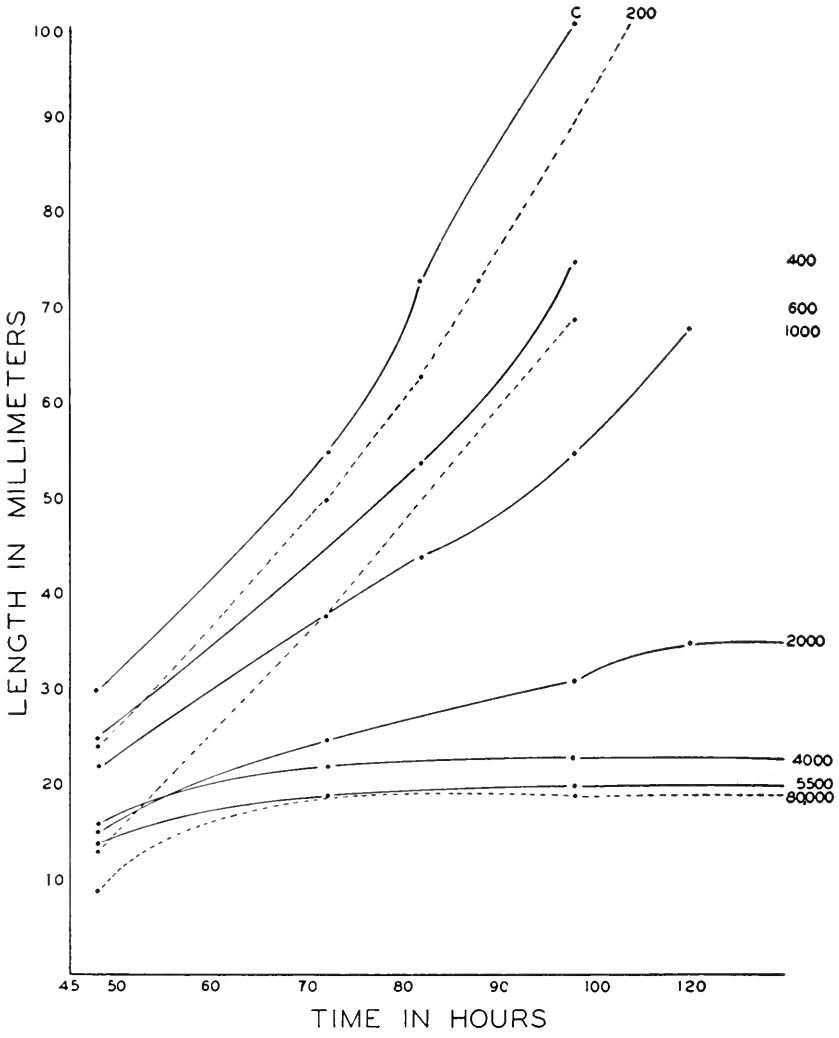


FIG. 1. Growth curves of primary roots after doses of neutrons up to 80,000 n

This was one case where a dose of neutrons sufficient to kill the plant had less effect on one kind of tissue than a killing dose of X-rays if these results with neutrons are compared with those of Smith and Kersten (8)

with X-rays. When one group of corn seedlings radiated with 600 n was compared with another radiated with 6000 r, it was found that the neutrons caused retardation in the length of the primary roots and delay in the appearance of the laterals, while there was no appreciable difference between the X-rayed seedlings and the controls. This indicates a neutron to X-ray ratio of less than unity, as was also found by other authors including Zirkle *et al.* (3) who used wheat seedlings and drosophila.

Observation of the adventitious roots was useful in the study of differences between the effects of fast neutrons and lower energy neutrons (Table I). A difference appeared when a comparison was made between the percentages of adventitious roots which were present after radiation doses of 2000–5500 n. With doses near such a level no lateral roots ap-

TABLE I

Effects of Fast Neutrons and Lower Energy Neutrons on the Adventitious Roots of Corn Seedlings

Dose	Length of Adventitious Roots		Per Cent of Plants with Adventitious Roots	
	Fast Neutrons	Lower Energy Neutrons	Fast Neutrons	Lower Energy Neutrons
n	*mm.*	*mm.*		
1000	40		100	
2000	15	14	100	83
4000	5	5	54	33
5500	5	2	52	6
15000	5		24	
26000	5		23	
80000	4		17	

peared at any time, and measurements of the primary roots showed little difference between 5500 and 80,000 n.

Effects on Lateral Roots. While the effects of neutrons on adventitious roots were apparent after comparatively large doses, the lateral rootlets growing out from the primary root indicated the neutron sensitivity within the range of about 100 to 2000 n. Martius (9) found that the difference between the time of appearance of lateral roots on controls and on X-rayed bean seedlings was proportional to the amount of radiation. Russell (5) found this to hold true for corn seedlings. There is apparently a tendency for the laterals on normal seedlings to appear when the primary root has reached a certain length.

A preliminary experiment was undertaken to investigate the possibility of stimulating plant growth by small doses of neutrons. Kersten *et al.* (10) had been able to stimulate corn growth with low doses of X-rays. Three

tubes of corn were irradiated with 53–160 n. The average length of the primary roots, 102 hours after germination, was 109 mm. for 19 control

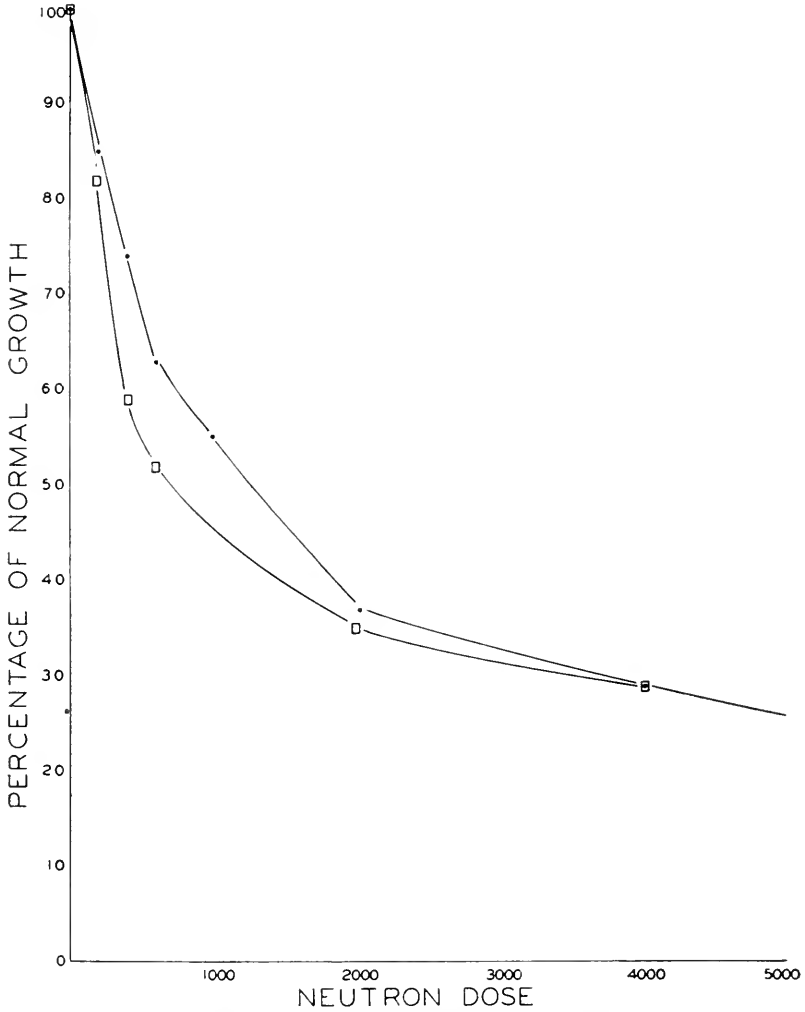
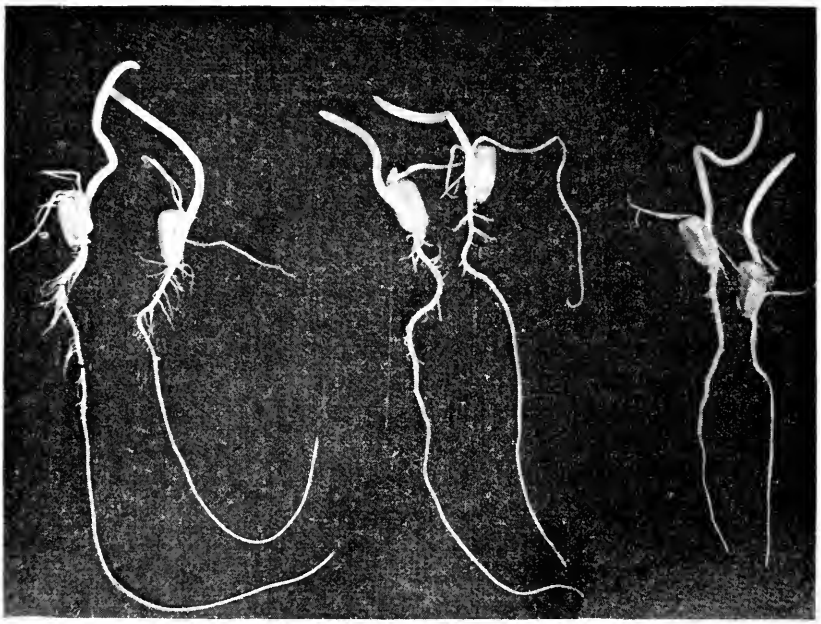


FIG. 2. Comparison of growth of primary roots after irradiation with fast neutrons and after lower energy neutron irradiation. ● = fast neutrons. □ = lower energy neutrons.

plants and 113 mm. for 64 irradiated plants. While this difference in the primary roots, taken alone, was not large, the picture changed when the

laterals were considered. In the control group, 5 per cent of the plants had lateral roots, while 42 per cent of the irradiated group had reached this stage of development. This work must be repeated with special precaution to make certain that the appearance of stimulation was not the result of some unknown factor interfering with the lateral roots of the controls.

Comparison of the Effects of Fast and Lower Energy Neutrons. When equal doses (according to measurement by the 100 r chamber) of the two qualities of neutrons were used, the primary roots subjected to the lower



A B C

FIG. 3. Effects of two qualities of neutrons on root development of corn. A = Controls. B = Radiated with 400 n (fast neutrons). C = Radiated with 400 n (lower energy neutrons).

energy neutrons were more damaged,than those subjected to fast neutrons within the range 200 to 2000 n (Fig. 2). When the time of appearance of the lateral roots of the two groups was compared, a dose as low as 100 n showed a difference between the two qualities of neutrons. When counts were made of both groups, 98 hours after germination, 70 per cent of the seedlings of the fast neutron group had laterals, while only 46 per cent of the lower energy group had them. When the two 400-n groups were examined 82 hours after germination, 32 per cent of the fast neutron group had laterals, while none was present in the others. The photograph

(Fig. 3) was taken a little later when the laterals were just beginning to appear in the lower energy group.

In the groups receiving 2000 n, the average length of the primary roots at the time of death was 31 mm. in the lower energy neutron group while it was 35 mm. in the fast neutron group. This difference in sensitivity is amplified when the record of the laterals is considered: 119 hours after germination 21 per cent of the fast neutron group had laterals, while none had appeared as yet in the lower energy group; 28 hours later, the former group had 100 per cent laterals, and laterals had appeared in 32 per cent of the latter group.

The difference in the effects of the two qualities of neutrons on adventitious roots of seedlings which had received doses from 2000 to 5500 n can be seen in Table I. While reduction in average length was nearly the same after both qualities of radiation, the lower energy neutrons significantly reduced the percentage of seedlings producing these roots.

The differences in effect of the two qualities of radiation may possibly have been due to a greater sensitivity of the 100 r chamber to fast neutrons. If this was true, the material radiated with lower energy neutrons would have actually received a greater dosage. No experiments have been done and no evidence was found in the literature that makes it possible to evaluate this hypothesis.

Other workers have found indications that living material is more sensitive to lower energy neutrons than to fast neutrons. Jennings and Garner (11) found evidence of this specific sensitivity in the case of certain bacteria. Stone (12) reported that to produce a minimum threshold skin reaction, larger doses were required when fast neutrons were used than when lower energy neutrons were used.

If the sensitivity of the 100 r chamber is the same for both qualities of neutrons, the results obtained with corn indicate that lower energy neutrons are more effective in retarding various phases of root development than are fast neutrons.

SUMMARY

1. Neutron radiation of dry seed of Zea mays affected the length of the primary root, the time of appearance of lateral roots, and the character of the adventitious roots.

2. Each type of response to radiation showed best within its own special range of dosage.

3. Small doses of fast neutrons appeared to have some stimulating effect.

4. Adventitious roots were more damaged by lethal doses of X-rays than by lethal doses of neutrons.

5. Corn seedlings were affected more by lower energy neutrons than by

fast neutrons when irradiated with equal doses according to the 100 r chamber.

REFERENCES

(1) Marshak, A., *Proc. Soc. Exptl. Biol. Med.*, **41**, 176 (1939).
(2) Thoday, J. M., *J. Genetics*, **43**, 189 (1942).
(3) Zirkle, R. E., Aebersold, P. C., and Dempster, E. R., *Am. J. Cancer*, **29**, 556 (1937).
(4) Gray, L. H., and Read, J., *Brit. J. Radiol.*, **15**, 72 (1942).
(5) Russell, M. A., *Plant Physiol.*, **12**, 117 (1937).
(6) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(7) Collins, G. N., and Maxwell, L. R., *Science*, **83**, 375 (1936).
(8) Smith, G. F., and Kersten, H., *Plant Physiol.*, **17**, 455 (1942).
(9) Martius, H., *Fortschr. Gebiete Röntgenstrahlen*, **32**, 361 (1924).
(10) Kersten, H. J., Miller, H. L., and Smith, G. F., *Plant Physiol.*, **18**, 8 (1943).
(11) Jennings, R. K., and Garner, J. M., Jr., Chapter 5.
(12) Stone, R. S., in "Medical Physics", edited by Otto Glasser, The Year Book Publishers, Inc., Chicago (1944), p. 814.

Chapter 8

THE EFFECT OF NEUTRON RADIATION ON SERUM ALKALINE PHOSPHATASE ACTIVITY

By FRANCIS E. REINHART

The phosphomonoesterases, widely distributed as they are in the plant and animal kingdoms, have been the subject of numerous investigations concerned with the elucidation of their functions and physiological significance. Much of this attention has been directed toward these enzymes in respect to the changes they may undergo during a variety of pathological conditions. Thus, liver damage and diseases involving the bone (rickets, Paget's disease, hyperparathyroidism, etc.) bring about an elevation in the blood serum content of alkaline phosphatase. The activity of acid phosphatase in serum is likewise raised in prostatic carcinoma metastatic to the skeleton.

In view of the indications that neutron irradiation of living animals may result in disturbances in the normal bone processes (1, 2), and of the close relationship of serum alkaline phosphatase to the latter, an investigation of this enzyme in the blood serum of irradiated animals has been initiated. Further interest in this problem arises from the work of Iwatsuru and Nanjo (3) on rabbits subjected to X-rays. The leukopenia resulting from this type of irradiation was found to be accompanied by a rise in the activity of serum alkaline phosphatase. An increase in the activity of this enzyme in serum has also been observed following X-ray treatment in cases of chronic myeloid leukemia (4, 5).

In the present study the serum enzyme activity in animals before and after whole body irradiation with neutrons has been determined. These observations, which are of a preliminary nature, were made on rats, rabbits, and dogs, most of which served simultaneously for the purposes of other investigations being carried out in this Laboratory.

METHODS

Serum. All blood samples were centrifuged approximately one hour after removal from the animals. Phosphatase tests were performed on the serum samples within the next four hours. In the experiments with rats, blood was obtained by decapitation of female albino animals averaging about 200 g. in weight and maintained on a diet of "Fox Blox" and water. They were used at the same time for studies concerning the changes in spleen weight after irradiation (6).

Rabbit serum was prepared from blood (2 to 3 cc.) obtained by heart puncture. Male animals weighing about 6 pounds were employed; they had access at all times to Purina Rabbit Chow and water.

A description of the dogs from which serum samples were obtained will be found elsewhere (7).

Irradiation. The animals were exposed to whole body neutron radiation. The procedure used in this Laboratory has been described by Enns *et al.* (8).

Alkaline Phosphatase Estimation. The method of Huggins and Talalay (9) was used with minor modifications. One-fourth the amounts of test solution and reagents specified by these authors was used in flat-bottom colorimeter tubes (14 x 96 mm.) in conjunction with a Klett-Summerson photoelectric colorimeter. Blank and experimental determinations were identical except that no phenolphthalein phosphate was used in the buffer employed for the former.

In the case of rat serum, samples were made up to twice their original volume with distilled water immediately before use; in addition, some highly active samples required twofold dilution of the phenolphthalein color with distilled water before a reading could be made. One hour was used for incubation at 37°C. and activity is expressed in terms of one-hour units per 100 cc. of serum (9).

With sera of low activity (rabbit, dog) no dilution was required. An incubation time of two hours was employed and the activity is given in terms of two-hour units per 100 cc. (9).

The use of chloroform in the alkaline buffer-substrate solution as specified by Huggins and Talalay was found to yield values for normal rat serum which were somewhat low when compared with those found in the absence of this agent. The inhibition amounted to 13 to 16 per cent; no difference was found in the slopes of the unit curves for each solution nor in the pH of each after the usual proportion of serum had been added. This apparent inhibitory action of chloroform was not encountered in the work on rabbits. Since these observations were first made after most of the estimations on rat sera had been performed, recorded values in this study are based on the use of buffer-substrate solutions saturated with chloroform.

SERUM ALKALINE PHOSPHATASE ACTIVITY IN RATS

The serum alkaline phosphatase activity has been investigated in nine groups of female rats (Table I). The animals in eight groups were previously exposed to a single dose (56 n) of neutron radiation in Box No. 7 (8) while the remaining group of normal animals served as controls. Enzyme estimations were made on the serum from each animal at the time of sacrifice, which occurred on various days after the irradiation period.

The summarized data show that the serum phosphatase activity of rats irradiated under these conditions undergoes a decrease which is at a maximum near the fourth day after exposure and which, at that time, amounts to approximately 75 per cent of the normal level. The average values for the various groups indicate a subsequent increase to near-normal values by the seventh day; on the twentieth day levels somewhat lower than normal are found.

In connection with this observed decrease in enzyme activity, several additional determinations have been carried out. Serum samples were made by mixing normal rat serum (high activity) with serum from rats previously irradiated (low activity) in various proportions. Since the phosphatase values found for the mixtures were always essentially equal

TABLE I

The Effect of Neutron Radiation on Serum Alkaline Phosphatase Activity in Female Rats

Number of Animals	Days after Exposure*	Alkaline Phosphatase†	
		Range	Average
14	(normal)	63.4–137.8	97.9
3	1	51.3– 60.5	54.9
3	2	51.6– 66.0	58.3
10	4	16.3– 41.8	24.7
10	5	27.6– 75.8	45.8
9	7	50.5–120.5	89.1
10	10	76.0– 95.8	84.3
9	14	61.5–115.4	87.1
10	20	43.6–106.3	75.4

* Irradiation dose: 56 n. The period of irradiation was approximately one hour.
† One-hour units per 100 cc. serum (9).

to those calculated on an additive basis, there is no indication that the decrease found after irradiation is due to the presence of inhibitors or to a lack of activating agents.

This decrease, in the light of the observation made by Ross and Ely (10) that the food intake of rats irradiated under similar conditions is greatly diminished, appears to resemble that observed by Weil and Russell (11) and by Gould (12) in rats which were fasted for one or more days. The decrease in serum alkaline phosphatase after fasting has been confirmed in this study; a group of ten rats was fasted for 24 hours, after which time the average enzyme activity was found to be 29.9 units. This value corresponds to a decrease of approximately 70 per cent based on that found for the normal group.

SERUM ALKALINE PHOSPHATASE ACTIVITY IN RABBITS

Since the enzyme activity in normal rabbit serum is much lower than that found in rat serum, a two-hour incubation time was used and the activity expressed in two-hour units per 100 cc. serum. Several one- and two-hour experiments on individual samples of rabbit serum gave an average value of 0.56 for the ratio between the one- and two-hour units. For a direct comparison of rat and rabbit enzyme levels, therefore, it is necessary to multiply the recorded rabbit values by this factor.

The alkaline enzyme activity was determined in five normal rabbits and re-determined at intervals after exposure of the animals to a single dose of

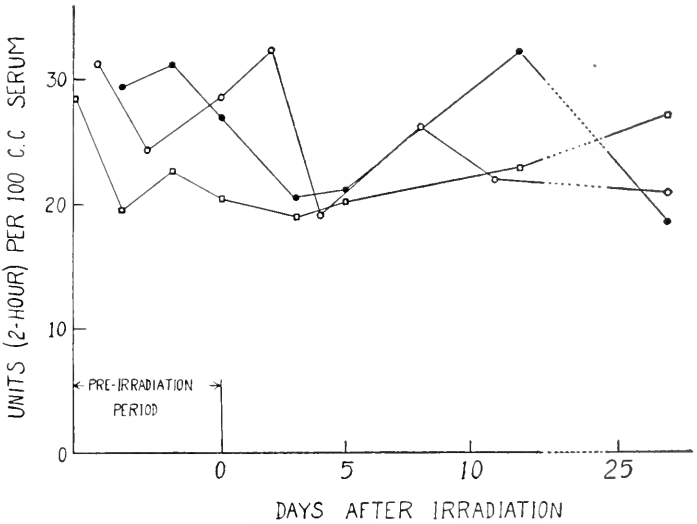


FIG. 1. Serum alkaline phosphatase activity in rabbits before and after irradiation with neutrons. Dose: 56 n in Box No. 4.

neutron radiation. Three of the animals were given 56 n in Box No. 4 while the remaining two received the same dose in Box No. 7. Under these conditions the rabbits in Box No. 4 were subjected to radiation of lower intensity but of higher energy (8) than those in Box No. 7. During the period of twenty-seven days following irradiation, no appreciable change in the enzyme level due to exposure in Box No. 4 could be found (Fig. 1). Of the two animals which were irradiated in Box No. 7, one showed an immediate drop in phosphatase activity which amounted to approximately 80 per cent on the tenth day (Fig. 2). The other rabbit underwent a smaller decrease which may be due, in part, to the fact that a less consistent base-line was encountered. In both cases, it is seen that

the activity was still below the pre-irradiation level seven weeks after the time of exposure.

In another experiment a rabbit was given a total dose of 112 n which consisted of two single doses of 56 n in Box No. 7 with an interval of ten days between exposures. Two weeks after the last dose had been given the serum phosphatase activity was found to be approximately 40 per cent of the pre-irradiation value.

A similar decrease in the serum enzyme level was observed when animals were subjected to larger amounts of radiation (300 n) given in small daily doses (10 n) in Box No. 4. Eight rabbits on which enzyme estimations

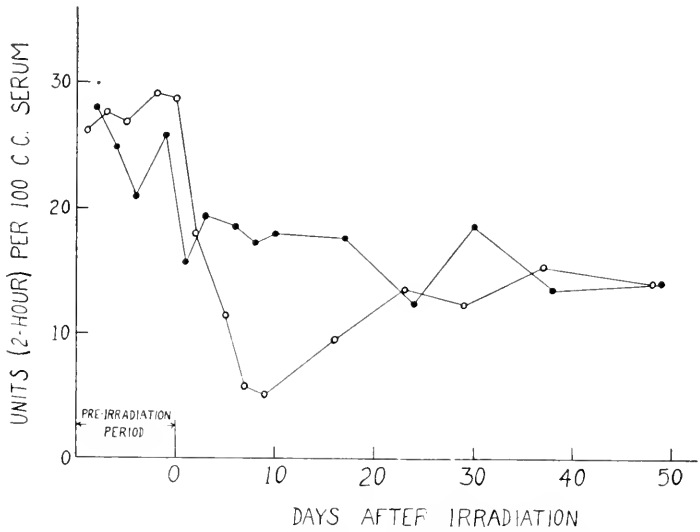


FIG. 2. Serum alkaline phosphatase activity in rabbits before and after irradiation with neutrons. Dose: 56 n in Box No. 7.

were performed 4 to 8 weeks after the completion of such a treatment possessed an average activity of 11.2 units (range, 7.0–17.8) while a group of ten normal rabbits gave an average of 25.1 units (range, 18.2–31.0).

During the course of this work on rabbit serum phosphatase activity, the serum of each of two animals for which alkaline enzyme data are shown in Fig. 2 was examined for possible effects of radiation on serum acid phosphatase activity. Estimations were carried out by the method of Huggins and Talalay (9) except that one-fourth the amounts of test-solution and reagents was used. Serum samples were diluted to twice their volume with distilled water just before use. Determinations made at intervals equal to those shown in Fig. 2 showed no significant changes in the acid

enzyme level in a period up to 17 days after exposure. Values for each animal in both the pre- and post-irradiation periods varied between 45 and 60 one-hour units per 100 cc. of serum.

SERUM ALKALINE PHOSPHATASE ACTIVITY IN DOGS

Several determinations of serum alkaline phosphatase activity were made on dogs before and after exposure to neutron radiation. These animals were those used in a study in this Laboratory on the effects of large doses of neutrons (7). The phosphatase data have been listed in Table II. It is seen that a dose of 400 n given over a period of four days resulted in an increased enzyme level; on the sixth day after completion of the irradiation

TABLE II

The Effect of Neutron Radiation on Serum Alkaline Phosphatase Activity in Dogs

Dog Number	Radiation*	Phosphatase Value†			
		Before Exposure	After Exposure		
			1 *day*	3 *days*	6 *days*
	n				
1	400	4.6			22.0
2	400	5.4			15.3
3	345	5.3	8.6		
	400			7.5	37.9
4	345	6.4	27.2		
	400			20.2	40.5

* The total dose consisted of 115 n on each of three successive days; and a dose of 55 n on the fourth day. Box No. 4 was used (8).

† Two-hour units per 100 cc. serum.

the activity in serum may reach a value which is 3 to 6 times that found before exposure. It is to be noted that the severe irradiation procedure here employed caused the death of three of the four animals in 6 to 8 days; dog No. 4 was killed on the seventh day (7).

DISCUSSION

The preliminary nature of the data presented in this study warrants few definite conclusions. It is possible, however, to point out several aspects which appear to be of interest in connection with the further investigation of the effects of neutrons on serum alkaline phosphatase activity. If the enzyme decrease in rats, as shown in Table I, is considered, it is found to

follow rather closely the decrease in food intake as observed by Ross and Ely (10) in rats irradiated under similar conditions. It can be pointed out, on the basis of the results obtained by Weil and Russell (11) and by Gould (12) on the effects of fasting, that a lack of fat absorption in rats would be expected to result in a lowered alkaline phosphatase activity in serum. Since the food intake data as well as other studies (10) point to a disruption in food assimilation after irradiation and since this disturbance is closely associated with the lowered enzyme activity, it is considered probable that the latter may be largely, if not wholly, due to this one factor.

In respect to the effects of neutron irradiation on the enzyme level in the rabbit, further investigation will be necessary before any conclusions can be made. The limited data, however, do indicate that irradiation employed under conditions which effect a decrease in the serum enzyme activity in the rat may result in a similar but more prolonged decrease in the rabbit enzyme. On the basis of the different energy values assigned to the radiation in Box No. 4 and Box No. 7 (8), it is further indicated that the enzyme decrease found in rabbits using a dose of 56 n at the lower energy levels may not occur when the same dose of higher energy neutrons is employed. It is evident that these points require further investigation.

A rise in the alkaline phosphatase activity in the serum of rabbits after exposure of the head or abdomen to X-rays (approximately 600 r) was observed by Iwatsuru and Nanjo (3); this rise was attributed by these authors to release of the enzyme by destroyed leukocytes. It is of interest to note that Wachstein (13), using a modified Gomori technique, could detect no alkaline phosphatase activity in the blood cells of the normal dog, although many of the polymorphonuclear leukocytes in rabbit blood showed strong activity. It would appear that destruction of leukocytes could not account for the increase in serum enzyme activity found in neutron-irradiated dogs employed in the present study. This increase, whether it be due to one or many factors, is not unusual in view of the severe bodily damage (7) suffered by these animals.

SUMMARY

The activity of alkaline phosphatase in the blood serum of rats, rabbits, and dogs has been determined before and after exposure of the animals to whole body neutron radiation.

When a dose of 56 n was employed, rats showed a 75 per cent decrease in the serum enzyme level on the fourth day; essentially normal values were found one week after exposure.

Limited data indicate that rabbits when irradiated under the same conditions may show a decreased serum enzyme activity; under these conditions, no significant changes were noted in the activity of serum acid phosphatase.

Dogs subjected to a lethal dose of neutron radiation possessed an increased serum alkaline phosphatase activity. The levels on the sixth day after completion of irradiation with 400 n were three to six times as high as the pre-irradiation levels.

REFERENCES

(1) YAMASHITA, H., *Gann*, **31,** 629; German Abstract, 654 (1937).
(2) LAWRENCE, J. H., AND TENNANT, R., *J. Exptl. Med.*, **66,** 667 (1937).
(3) IWATSURU, R., AND NANJO, K., *Biochem. Z.*, **300,** 429 (1939).
(4) IWATSURU, R., AND NANJO, K., *Biochem. Z.*, **300,** 422 (1939).
(5) ALBERS, D., *Z. ges. exptl. Med.*, **105,** 155 (1939).
(6) ELY, J. O., ROSS, M. H., AND GAY, D. M., Chapter 20.
(7) ROSS, M. H., AND ELY, J. O., Chapter 19.
(8) ENNS, T., TERRILL, H. M., AND GARNER, J. M., JR., Chapter 3.
(9) HUGGINS, C., AND TALALAY, P., *J. Biol. Chem.*, **159,** 399 (1945).
(10) ROSS, M. H., AND ELY, J. O., Chapter 17.
(11) WEIL, L., AND RUSSELL, M. A., *J. Biol. Chem.*, **136,** 9 (1940).
(12) GOULD, B. S., *Arch. Biochem.*, **4,** 175 (1944).
(13) WACHSTEIN, M., *J. Lab. Clin. Med.*, **31,** 1 (1946).

Chapter 9

THE EFFECT OF NEUTRON RADIATION ON THE PEROXIDASE AND CATALASE ACTIVITY OF BONE MARROW

By GLADYS E. WOODWARD

Bone marrow from the leg bones of rabbits and dogs, removed at death of animals treated in connection with other phases of this radiation project (1), has been investigated for its peroxidase and catalase activity.

The determination of peroxidase is complicated by the presence of hemoglobin and catalase in whole marrow. Therefore, in the present study, washed marrow has been used, the washing eliminating all of the hemoglobin and that part of the catalase which is present in the red blood cells. The method of Schwimmer (2), which measures peroxidase by its catalytic effect on the reaction between H_2O_2 and KI, has been applied to the washed bone marrow. Since catalase also acts upon H_2O_2, its presence interferes with the estimation of peroxidase by reducing the concentration of H_2O_2 available for the peroxidase reaction. Unless catalase is present in considerable excess, however, the error in the peroxidase value is only slight.

PROCEDURE

Marrow was removed from one to three bones, weighed and mixed with 9 parts of water to give a 1:10 suspension. By gentle agitation, the red blood cells present were easily broken up and the hemoglobin removed in the supernatant fluid by centrifugation. The washing was repeated until the marrow sediment became free of hemoglobin. The 1:10 suspension of washed marrow was then homogenized by submitting to intense vibrations of 10.5 kc. provided by a Pierce magnetostriction oscillator of the Chambers and Flosdorf type (3). During treatment, 5 to 7 cc. aliquots, which were subsequently mixed, were placed in a 10 cc. Pyrex beaker which was fixed at about 1 mm. above the top of the vibrating nickel tube. Water, contained in a water jacket, was the medium between nickel tube and beaker. The duration of treatment was from 5 to 10 minutes depending upon consistency of the tissue. The homogenized mixture was used for the peroxidase and catalase estimations.

In the estimation of peroxidase, $\frac{1}{4}$ the quantities of the Schwimmer (2) method was used. To a mixture of 10 cc. reagent (containing 0.027 M KI, 0.001 M $Na_2S_2O_3$, 0.1 per cent starch, and 0.02 M acetate buffer of pH 4.7) and varying amounts of homogenized marrow suspension diluted to 2.25

cc., at 25°, was added 0.25 cc. of 0.9 per cent H_2O_2; the time required for appearance of the starch-iodine color was noted. The amount of enzyme which caused appearance of this color in one minute was regarded as one unit of peroxidase. In order to make a correlation between marrow samples, which vary considerably in fat and water content, nitrogen determinations were made (by the Microchemical Department), and the units of peroxidase calculated per mg. nitrogen.

Catalase was determined under the same conditions of pH, temperature and concentration as were used for the peroxidase determinations, in order to determine the relative activity of the two enzymes. Warburg manometers with simple, single side-arm flasks were used. In the side-arm was placed 0.05 cc. of 0.9 per cent H_2O_2, and in the main part of the flask 0.4 cc. of 0.1 M acetate buffer of pH 4.7, 0.05 to 0.4 cc. of marrow, and enough water to make a total of 2.5 cc. The amount of oxygen liberated was read on the manometers at different time intervals. The activity was calculated in terms of H_2O_2 decomposed per minute per mg. nitrogen.

RESULTS

The values obtained in the washed marrow from neutron irradiated and untreated rabbits and dogs are reported in Table I. The data show that when the radiation has affected the animal to such an extent as to bring it near death or cause death, no peroxidase was found in the bone marrow investigated. In the case of rabbit No. 2, a very low peroxidase value was found. This animal had been near death toward the end of the radiation treatment but on cessation of the radiation treatment was recovering. In animals whose health was apparently not affected by the radiation and in untreated animals the peroxidase values were much higher and of the same magnitude. No correlation can be made at this time between the amount of radiation and the diminution of peroxidase in the bone marrow because of the few animals studied.

Evidence was obtained that little, if any, peroxidase was lost from the marrow tissue during removal of the hemoglobin by washing. A peroxidase value and a hemoglobin analysis was obtained on the wash water and the peroxidase value compared to that produced by pure hemoglobin. The hemoglobin content of the wash water accounted for the peroxidase value within reasonable limits.

When zero peroxidase was reported, in Table I, the experimental values were actually negative. This was due to a retardation of the reagent blank time because of a lowering of the peroxide concentration which was a result of destruction of peroxide by catalase. By measuring the amount of oxygen liberated under identical conditions, however, the change in the peroxide concentration could be calculated. When a correction for this

TABLE I

Peroxidase and Catalase Activity of Bone Marrow from Irradiated and Untreated Animals

Animal	Peroxidase	Catalase	Radiation† Treatment	Days Since Last Radiation	White Blood Cell Count	Animals' General Condition
	units per mg. N*	*moles H_2O_2 × 10^6 decomposed per mg. N per min.*	*n*		*per cmm.*	
Irradiated rabbit						
1	0	16.8	56.4 × 3 = 169.2	11	5,300	near death
2 sample A	2.2	13.9	56.4 × 2 = 112.8 10 × 7 = 70 Total = 182.8	76	—	recovering
2 sample B	3.1	—				
3	13	22.3	14 × 21 = 294	32	9,400	excellent
4	11	10.1	11.28 × 30 = 338.4	61	13,000	"
5 sample A	5.3	8.8	11.28 × 30 = 338.4	35	9,600	"
5 sample B	16	13.9				
Untreated rabbit						
1	10	22.3	none		—	
2 sample A	10	10.4	"		14,550	
2 sample B	8	7.3				
3	8	12.7	"		16,850	
Irradiated dog						
1	0	4.6	115 × 3 = 345 55 × 1 = 55 Total = 400	8	300	died
2	0	4.6	"	8	1,100	died
3	0	6.6	"	6	50	died
4	0	7.2	"	7	150	near death
5	0	—	1.7 × 329 = 559	0	2,350	died
6	48	—	0.110 × 312 = 34.32	2	8,700	good
Untreated dog						
1 femur	16	—	none		14,650	
1 humerus, A	59	—				
1 humerus, B	78	—				
2	175	40.6	none		10,050	
3	52	14.8	none		10,700	

* A unit of peroxidase represents the amount of material which uses 1×10^{-6} moles of H_2O_2 in 1 minute.

† For example, a radiation treatment expressed as "56.4 × 3 = 169.2", means 56.4 n was the dose each time, administered 3 times, totaling 169.2 n.

change in concentration was applied, the negative values approached zero, as reported in Table I. The only other value subject to the catalase error is that for irradiated rabbit No. 2, which would be slightly higher if corrected.

The catalase activity of the washed marrow from the irradiated rabbits studied was as high as that of the untreated rabbits. This was true even in the case of irradiated rabbit No. 1, where the peroxidase activity had been reduced to zero. In the case of dogs, however, the values for the irradiated animals were considerably lower than for the two untreated dogs studied.

DISCUSSION

The existence of peroxidase in animal tissues has been doubted by many authors. Bancroft and Elliott (4), however, gave evidence of the probability of its occurrence in small amounts in spleen and lung. Dempsey (5) reported it in tissue of thyroid gland, but Glock (6) gave evidence that this activity could be accounted for by the hemoglobin content. It is known to occur in considerable quantity in the animal, however, in white blood cells of the granulocyte series (7) and in milk (8). The finding of considerable amounts of peroxidase in bone marrow in the present study cannot be due to the presence of hemoglobin, since this was completely removed by washing. Its presence is most probably due to the presence of white blood cells which are normally found in bone marrow.

Radiation with X-rays and with neutrons is known to produce an early and sharp diminution in the number of white cells present in the blood (9, 10) and in the bone marrow (11, 12). It is reasonable to suppose, therefore, that a lack of white blood cells in the marrow accounts for the absence of peroxidase in the six cases reported in Table I. It will be noted that in five of these cases the white cell count in the blood was exceedingly low. The presence of this small number of white cells in the blood may possibly be accounted for by production in other marrow which was not investigated in the present study.

SUMMARY

Peroxidase has been found to be absent from the marrow of leg bones of rabbits and dogs at or near death as a result of neutron radiation. In animals whose health was not materially affected by the radiation, the peroxidase values were in the same range as those found for untreated animals.

Catalase activity of the marrow from rabbits did not appear to have been affected, while in dogs the activity appeared to be decreased as a result of the radiation.

REFERENCES

(1) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(2) Schwimmer, S., *J. Biol. Chem.*, **154,** 487 (1944).
(3) Chambers, L. A., and Flosdorf, E. W., *Proc. Soc. Exptl. Biol. Med.*, **34,** 631 (1936).
(4) Bancroft, G., and Elliott, K. A. C., *Biochem. J.*, **28,** 1911 (1934).
(5) Dempsey, E. W., *Endocrinology*, **34,** 27 (1944).
(6) Glock, G. E., *Nature*, **154,** 460 (1944).
(7) Osgood, E. E., and Ashworth, C. M., "Atlas of Hematology," J. W. Stacey, Inc., San Francisco (1937), p. 106.
(8) Elliott, K. A. C., *Biochem. J.*, **26,** 10 (1932).
(9) Lawrence, J. H., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22,** 124 (1936).
(10) Ross, M. H., and Ely, J. O., Chapter 18.
(11) Ely, J. O., Ross, M. H., and Gay, D. M., Chapter 20.
(12) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66,** 667 (1937).

CHAPTER 10

EFFECT OF NEUTRONS ON THE RIBONUCLEINASE IN RABBIT BLOOD AND TISSUES

BY CHARLES A. ZITTLE

Variations in the ultraviolet light absorption of cells in biopsy specimens after irradiation with therapeutic doses of X-rays and gamma rays found by Mitchell (1) led to the conclusions that there is an accumulation of ribonucleic acid products in the cytoplasm and a cessation of synthesis of desoxyribonucleic acid in the nucleus. Disturbances in the metabolism of both ribonucleic and desoxyribonucleic acids on irradiation with ionizing rays was brought out in later papers by Mitchell as well (2, 3), effects which were considered to be due to inactivation of catalyst molecules, presumably components of enzyme systems. Studies by Marshak (4) also suggest an involvement of nucleic acids in the effects of X-rays. By means of radioactive phosphorus (P_{32}) in tracer studies a shift of the phosphorus from the cytoplasm to the nucleus in lymphoma cells after irradiation with X-rays was demonstrated. This shift is probably correlated with changes in the metabolism of nucleic acid since it contains phosphoric acid.

The above observations suggested that enzymes involved in the metabolism of nucleic acid be investigated in animals treated with neutrons, since the fundamental biologic effects of X-rays and neutrons have been assumed (5) to be the same and dependent on the degree of ionization produced within tissue cells. The ionizing rays, however, may affect directly the nucleic acids and these in turn influence enzymes (i.e., dehydrogenases) (6).

The present studies have been made on ribonucleinase, the enzyme which catalyzes the depolymerization of ribonucleic acid with the formation of mononucleotides (7), since a convenient manometric procedure was available (8) for its determination, and since its content in the blood and tissues of normal rats and rabbits has been reported (9). The content of this enzyme in the tissues of irradiated rabbits is reported herein.

Studies are desirable with the enzymes which hydrolyze desoxyribonucleic acid as well. The enzyme which depolymerizes desoxyribonucleic acid has been purified (10) but hydrolysis of nucleic acid by this enzyme apparently stops at the tetranucleotide stage (11). Further hydrolysis to mononucleotides and nucleosides is brought about by a phosphoesterase from calf intestinal mucosa and other tissues (12-16), which is not highly specific,

however, since it hydrolyzes ribonucleic acid also. Studies on this enzyme have been initiated in this Laboratory (17) because of its possible relation to the irradiation problem. A method has been described (17, 18) for distinguishing this enzyme from ribonucleinase when ribonucleic acid is the substrate. In the blood and tissues studied herein the predominant enzyme is ribonucleinase (18).

EXPERIMENTAL

Ribonucleinase was crystallized by the method of Kunitz (19) and a stock solution prepared (9).

Several commercial brands of yeast ribonucleic acid were employed as the substrate. The method of purification and the desirable properties of nucleic acid to serve as substrate have been described (9, 20, 21).

Method of Assay of Ribonucleinase. The assay method is based on the liberation of CO_2 from a bicarbonate solution by the acidic mononucleotides and intermediate products which are formed by the action of the enzyme. The components of the test system were the following: 1.0 cc. of 0.1 M $NaHCO_3$, 160 mg. of nucleic acid in solution at pH 7.5, and appropriate amounts of the crystalline enzyme or the material under test. The total volume of reactants was in most cases 3.5 cc. The usual Warburg equipment was employed. The crystalline enzyme or the nucleic acid was placed in the side arm of the flasks and the system thoroughly gassed at 37° with a 5 per cent CO_2-95 per cent N_2 mixture. After equilibrium was reached, the reactant in the side arm was tipped in and the manometers read at intervals. The pH attained by this system is 7.5, the optimum for ribonucleinase (19, 8). Standardization of the assay procedure with crystalline ribonucleinase has been described (9).

In the application of the above test to buffered biological fluids and extracts the CO_2 retained by the buffers must be determined for accurate results. This was done by determining the CO_2 evolved from the buffered system by citric acid in comparison with an unbuffered system. Details of the procedure have been described (9).

The blood for the assays was obtained by heart puncture from male rabbits and collected in a tube containing 1.0 to 2.0 cc. of 2 per cent sodium oxalate to 10 cc. of blood. The oxalate did not affect the results with crystalline ribonucleinase. A precipitate, probably calcium oxalate, formed with the nucleic acid. The blood cells were diluted with 0.85 per cent NaCl to the volume of the blood from which they were taken. The bone marrow was obtained (0.5 g. sample) from the end portion of the femur of the hind leg. The minced tissue was suspended in water (100 mg./1.0 cc.) and broken up in a homogenizer. Treatment in a Pierce magnetostriction oscillator, frequency about 10,000 cycles per second, for 5 minutes effectively dispersed the bone marrow but was not satisfactory for the other tissue.

The neutron source, method of measurement, method of exposure of the animals, etc. are described by Enns *et al.* (22).

RESULTS

The ribonucleinase content of the blood and tissues of normal and neutron-treated rabbits is shown in Table I.

TABLE I

The Ribonucleinase Content of the Blood, Spleen and Bone Marrow of Neutron-Treated Rabbits

Designation of Rabbit	Irradiation Treatment 55 n day Total n	Time After Irradiation	Ribonucleinase Content		
			Blood	Spleen	Bone Marrow
		days	*cmm./cc./hr.*	*cmm./mg. wet wt./hr.*	*cmm./mg. wet wt./hr.*
Normal*	none	—	24 to 144	2.9 to 9.0	0.8 to 2.4
2	220	1	39	3.9	3.2
3	220	4	102	2.0	0.1
4	220	2	30	2.4	0.1
5	220	5	0	2.3	0.3
6	495	2	—	4.0	0.6
8	275	1	87	2.7	0.5
9	275	5	0	1.3	0.2

* 3 to 8 rabbits were used for determining the range of normal values.

DISCUSSION

The data for normal rabbits, as well as for rats, have been discussed (9) and compared with the data obtained by Bain and Rusch (8) for the ribonucleinase in the pancreas and spleen of the rat.

The ribonucleinase content of the spleens of the neutron-treated rabbits is on the low side of the normal range but the deviation is not significant. The ribonucleinase in the bone marrow of the neutron-treated rabbits, however, has decreased significantly, with the exception of No. 2 rabbit, being only three-quarters to one-eighth of the lowest value in the normal range. This decrease is probably correlated with the decreased production of the cellular elements of the blood by the bone marrow after irradiation. The measurements of the blood ribonucleinase in normal animals show that this enzyme accompanies the cellular elements of the blood (9). In the case of the ribonucleinase in the blood of the neutron-treated animals the results with four of the animals were within the normal range. The blood of two rabbits (Nos. 5 and 9) contained only a negligible amount of this enzyme. This finding was not surprising for rabbit No. 9, for the

cellular elements of the blood were greatly reduced and the animal died from the treatment. In the case of both animals, Nos. 5 and 9, a longer time (5 days) had elapsed before the blood samples were taken than with the other animals.

In neither the bone marrow nor the blood does the reduction in the ribonucleinase appear to be a primary effect on the enzyme but appears more likely to be in consequence of the reduction in the number of blood cells in the blood or at their site of formation in the marrow.

Ionizing radiations exert their effect on enzymes (23) either by direct ionization within the molecule, or indirectly by ionization within the aqueous environment. Inactivation by the latter mechanism is presumably by the intervention of an intermediate agent formed by ionization or excitation of the water. The direct effect can only be demonstrated with concentrated solutions or dry enzymes (23). In the case of large molecules like viruses direct inactivation was readily demonstrated with a 1 per cent virus solution (23), and it was noted that an ionization in the water has a rather small probability of causing inactivation of the virus, presumably because the activated water can lose its energy on contact with the virus particle without invariably leading to inactivation of the virus particle. The desoxyribonucleic acid, which occurs in the nucleus of cells, might be expected to respond to irradiation like a virus since it has a high molecular weight and high density (15).

It has been found that ionizing rays which hardly influence respiration or glycolysis very strongly influence cellular division (growth) (24). Cells that are dividing slowly are least vulnerable to ionizing rays, presumably having time to eliminate toxic products before the sensitive process of cell division occurs. These considerations suggest that the nucleic acid in the nucleus of the cell may be the point of vulnerability of living systems to X-rays and neutrons.

X-rays and neutrons might cause a depolymerization of the nucleic acid to tetra- and mononucleotides. It has been pointed out (6) that, since nucleic acids and their hydrolytic products are inhibitory to the cellular dehydrogenases, any influence changing the ratio of nucleic acid to the more diffusible (i.e., more widely reactive) hydrolytic products might profoundly alter the economy of the cell.

In attempt to demonstrate an effect of neutrons on nucleic acid a 10 per cent solution of ribonucleic acid was treated with 750 n. A slight depolymerization occurred, measured by precipitation with the uranium reagent (25), but it was not sufficient to affect the ability of the nucleic acid to serve as a substrate for ribonucleinase. The molecular weight of ribonucleic acid is, however, much less (26, 27) than that of desoxyribonucleic acid (15). Irradiation may not have to cause so great a change

in the desoxyribonucleic acid as discussed above to disturb its function in the cell. The studies (28) which have shown that desoxyribonucleic acid is probably the factor determining the specific type of the pneumococci suggest a high degree of specificity of the desoxyribonucleic acids. Since chemical studies so far have not revealed any differences, this specificity may well reside in physical arrangement of the molecule. A specific orientation of the molecule might readily be disturbed by irradiation with consequent deep seated disturbance of cellular division.

The large amount of evidence that the nucleus of the cell and the nuclear (desoxyribo-) and cytoplasmic (ribo-) nucleic acids are closely involved in the effects of X-rays and neutrons on the cell emphasizes the importance of research on these constituents. Hypothetical mechanisms for the interaction of ionizing rays and the nucleic acids suggest paths to be investigated.

SUMMARY

The ribonucleinase content of the blood, bone marrow and spleen of neutron-treated rabbits was determined and compared with the values obtained with normal rabbits. Significant reductions occurred in the bone marrow and in a few cases extremely low values were found in the blood. In no case, however, did the effect appear to be a primary one.

REFERENCES

(1) Mitchell, J. S., *Brit. J. Exptl. Path.*, **23**, 296, 309 (1942).
(2) Mitchell, J. S., British Empire Cancer Campaign, 21st Annual Report, p. 62 (1944).
(3) Mitchell, J. S., *Brit. J. Radiol.*, **16**, 339 (1943).
(4) Marshak, A., *J. Gen. Physiol.*, **25**, 275 (1941).
(5) Warren, S., *Physiol. Rev.*, **24**, 225 (1944).
(6) Zittle, C. A., *J. Biol. Chem.*, **162**, 287 (1946).
(7) Loring, H. S., and Carpenter, F. H., *J. Biol. Chem.*, **150**, 381 (1943).
(8) Bain, J. A., and Rusch, H. P., *J. Biol. Chem.*, **153**, 659 (1944).
(9) Zittle, C. A., and Reading, E. H., *J. Biol. Chem.*, **160**, 519 (1945).
(10) McCarty, M., *J. Gen. Physiol.*, **29**, 123 (1946).
(11) Fischer, F. G., Boettger, L., and Lehmann-Echternacht, H., *Z. physiol. Chem.*, **271**, 246 (1941).
(12) Klein, W., *Z. physiol. Chem.*, **207**, 125 (1932).
(13) Klein, W., *Z. physiol. Chem.*, **218**, 164 (1933).
(14) Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.*, **149**, 369 (1943).
(15) Schmidt, G., Pickels, E. G., and Levene, P. A., *J. Biol. Chem.*, **127**, 251 (1939).
(16) Laskowski, M., and Seidel, M. K., *Arch. Biochem.*, **7**, 465 (1945).
(17) Zittle, C. A., *J. Franklin Inst.*, **241**, 379 (1946).
(18) Zittle, C. A., and Reading, E. H., *J. Franklin Inst.*, **242**, 124 (1946).
(19) Kunitz, M., *J. Gen. Physiol.*, **24**, 15 (1940).
(20) Zittle, C. A., *J. Biol. Chem.*, **160**, 527 (1945).
(21) Zittle, C. A., *J. Biol. Chem.*, **163**, 111 (1946).
(22) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.

(23) Lea, D., Smith, K. M., Holmes, B., and Markham, R., *Parasitology*, **36**, 110 (1944).
(24) Hevesy, G., *Rev. Modern Phys.*, **17**, 102 (1945).
(25) MacFadyen, D. A., *J. Biol. Chem.*, **107**, 297 (1934).
(26) Fletcher, W. E., Gulland, J. M., Jordan, D. O., and Dibben, H. E., *J. Chem. Soc.*, p. 30 (1944).
(27) Cohen, S. S., and Stanley, W. M., *J. Biol. Chem.*, **144**, 589 (1942).
(28) Avery, O. T., MacLeod, C. M., and McCarty, M., *J. Exptl. Med.*, **79**, 137 (1944).

Chapter 11

STUDIES OF PROTEOLYTIC ENZYMES IN BONE MARROW

By WILLIAM G. BATT and CHARLES A. ZITTLE

The object of this investigation was to study the presence of any proteolytic enzymes that may be present in bone marrow. If the evidence warranted it, a comparison was to be made between the proteolytic enzyme content of non-irradiated animals and animals which had been subjected to the influence of neutron radiation.

A preliminary study was made to determine the optimum pH range and the limiting concentration of substrate. The technic employed followed that developed by Linderstrom-Lang and Holter (1) and Weil and Jennings (2), but crystallized bovine plasma albumin was used as the proteinase substrate for the standardization experiments.

The results obtained when using minced rabbit kidney extracted with 60 per cent glycerol agreed with Weil's value for the optimum pH (7.5) but differed slightly in the limiting concentration of the substrate. It was found that the plateau began nearer 8 per cent rather than the lower value of 5 per cent.

After establishing these conditions, a study was begun to detect the presence of proteolytic enzyme in bone marrow. No attempt was made to separate the red and yellow portions, but a pooling of all the marrow was used. This was divided, however, into 2 portions, one being extracted with 60 per cent glycerol and the other with physiological saline solution. No appreciable difference was noted on comparing the values obtained from both extracts. In both cases they were negligible. Changes in concentration of bone marrow and increase in the time of reaction gave very little increased value. Changing the temperature from 25° to 37° was of no advantage.

With no apparent reaction between the bone marrow extract and bovine plasma albumin it was decided to change the substrate to d-l leucylglycylglycine. The results were similar to those obtained with the previous substrate.

In order to check the efficiency of extraction of the bone marrow a sample was obtained which had been sonicized by submitting it to intense vibrations of 10.5 kc. provided by a Pierce magnetostriction oscillator of the Chambers and Flosdorf type (3). This sample was incubated with d-l leucylglycyl-glycine at 37°C. but no appreciable difference was obtained as compared with the non-sonicized ones.

The substrate was again changed, this time to edestin and cysteine at a pH of 3.95, and both sonicized and ordinary samples of bone marrow extracts were examined. One series of tests was run as long as 7 days at 37°C. but no increase in reaction values was obtained.

A sample of bone marrow from a dog which had been subjected to neutron radiation was examined with this particular substrate, but the values were as negligible as those found on the bone marrow of normal animals.

SUMMARY

No evidence of proteolytic enzymes was found in either sonicized or non-sonicized samples of bone marrow from normal animals. A sample from an irradiated dog was also negative.

REFERENCES

(1) Linderstrom-Lang, K., and Holter, H., *Compt. rend. Lab. Carlsberg*, **19**, No. 4 (1931).

(2) Weil, L., and Jennings, R. K., *J. Biol. Chem.*, **139**, 421 (1941).

(3) Chambers, L. A., and Flosdorf, E. W., *Proc. Soc. Exptl. Biol. Med.*, **34**, 631 (1936).

CHAPTER 12

EFFECT OF NEUTRON IRRADIATION ON PROTEINS OF SONICIZED BONE MARROW AND BLOOD PLASMA OF RABBITS

BY LAURA E. KREJCI, JAMES L. LEITCH, AND LUCILE SWEENY

INTRODUCTION

Since neutron irradiation of rats (1) brings about a decrease in the leukocyte and erythrocyte counts indicative of some effects on the leukopoietic and erythropoietic systems, it was deemed advisable to determine whether or not neutron irradiation produces any changes in the proteins of bone marrow. Although considerable data are available in the literature on the cellular constituents of bone marrow, both normal and pathological, little is known concerning the protein constituents of this organ. Müller (2) and Keilhack (3) have reported data, obtained by salt precipitation methods, on the protein distribution in the bone marrow of rabbits (summarized in Table I).

Inspection of Table I indicates wide differences in the results obtained by Müller and Keilhack. Keilhack showed that only a portion of the fibrinogen in bone marrow is removed by the short period of extraction used by Müller. The values found for fibrinogen by Keilhack were obtained only when the buffered saline solution was left in contact with the ground bone marrow for at least 24 hours. These data must therefore be considered more reliable than those of Müller. However, both sets of results are open to the criticism that they are based apparently on the values found for the total protein of the extract and not of the original sample of bone marrow. It is therefore evident that the data in the literature are of questionable significance with regard to the protein distribution in bone marrow.

MATERIALS AND METHODS

Animals. Rabbits were chosen as the experimental animal because of the large amount of bone marrow obtainable and the greater ease with which both blood and bone marrow could be obtained. Four rabbits, referred to as A, B, C, and D, respectively, were used and kept under observation for periods ranging from 14 to 110 days. A fifth rabbit, E, was an extra animal that had previously been used in the laboratory as a source of normal blood and was not studied by the authors prior to the time of its use.

At intervals throughout the observation period, weight and hematological data were obtained on each animal. No significant changes were observed in the erythrocyte count and blood hemoglobin levels, so that these data

TABLE I

Data Compiled from Müller (2) and Keilhack (3) on the Nitrogen and Protein Distribution in Rabbit Plasma and in Bone Marrow

Constituents	Reference	Concentration g% or % Total Protein	Rabbit Plasma			Rabbit Bone Marrow		
			Minimum Concn.	Maximum Concn.	Average Concn.	Minimum Concn.	Maximum Concn.	Average Concn.
Total nitrogen	3	g%	0.927	1.261	1.121	0.360	0.957	0.622
Non-protein nitrogen	3	g%	0.024	0.074	0.045	0.054	0.204	0.110
Total protein	2	g%	3.096	5.928	4.628	1.919	3.869	2.909
	3	g%	5.456	7.731	6.722	1.689	5.125	3.195
Fibrinogen	2	g%	0.415	0.638	0.561	0.238	0.618	0.391
		%	—	—	12.1	—	—	13.4
	3	g%	0.194	0.663	0.296	0.450	2.144	1.095
		%	3.07	9.97	4.40	16.89	46.34	32.50
Euglobulin	3	g%	0.257	2.250	1.065	0.150	1.162	0.382
		%	3.70	30.40	15.84	2.96	22.66	11.89
Pseudoglobulin I	3	g%	0.513	1.206	0.776	0.113	0.413	0.222
		%	7.86	17.87	11.54	3.15	11.84	7.02
Pseudoglobulin II	3	g%	0.350	1.031	0.635	0.113	0.488	0.236
		%	4.70	15.50	9.45	3.14	12.00	7.78
Total globulin	2	g%	0.867	2.813	1.678	0.529	1.273	1.001
		%	—	—	36.2	—	—	34.4
	3	g%	1.527	3.473	2.476	0.413	2.062	0.840
		%	26.73	46.45	36.83	11.50	40.25	26.69
Albumin	2	g%	1.395	3.174	2.389	0.666	2.598	1.518
		%	—	—	51.6	—	—	52.2
	3	g%	3.211	4.875	3.950	0.713	2.137	1.260
		%	50.18	69.02	58.77	21.43	57.67	40.81

are not reported in detail. The data on weight, leukocyte counts and per cent lymphocytes during the first 25 days, which was the critical period in the experiment, have been graphically reported in Fig. 1, while complete data are summarized in Table II. The primary hematological changes

observed in the irradiated animals were a reduction in total white blood cell count and a decrease in the per cent of lymphocytes.

Radiation. Two rabbits, A and C, were irradiated on the 13th day with 135 n of neutrons in two hours by the cyclotron of the Biochemical Research Foundation under the supervision of the Physics Department. The details of this cyclotron, of the methods and conditions of irradiation, and of the characteristics of the neutron beam, have been described by Enns *et al.* (4). Three rabbits, B, D, and E, served as non-irradiated controls.

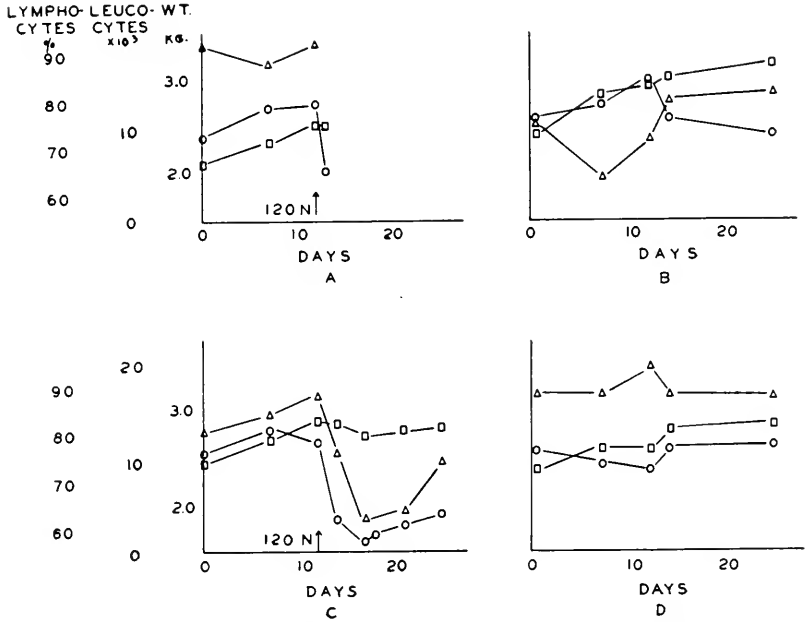


FIG. 1. Changes in body weight (□), total leukocyte count (○), and per cent lymphocytes (△) for irradiated rabbits, A and C, and non-irradiated rabbits, B and D.

Blood Studies. Plasma and serum of the rabbits were utilized to obtain indirect evidence of the condition of the animals and as a means of determining changes in the blood proteins on irradiation and on repeated bleedings. Both plasma and serum were examined by the electrophoretic technique of Tiselius (5), and the plasma was analyzed in addition for total and non-protein nitrogen by the micromethod of Kjeldahl. Blood (12 ml.) was drawn by cardiac puncture, after 24 hours' starvation; 7 ml. were converted to plasma, using 0.2 mg. of lithium oxalate per ml. as anticoagulant, and the remainder was converted to serum. For electrophoresis

TABLE II

Hematological, Plasma Protein and Bone Marrow Data

Rabbit	Neutron Dose	Day of Observation	Rabbit Weight	Hematological Data				Plasma Protein Data			Plasma Non-protein Nitrogen	Bone Marrow Data				
				Leukocytes	Lymphocytes	Erythrocytes	Blood Hemoglobin	Concn. Calcd. from Nitrogen Analyses	Refractive Increment (Electrophoresis)	Ratio $\frac{\Delta n \times 10^4}{g.\%}$		Total Weight of Bone Marrow	Protein Concn. Calcd. from Nitrogen Analyses	Refractive Increment (Electrophoresis)	Ratio $\frac{\Delta n \times 10^4}{g.\%}$	Protein Extracted per 100 g. Bone Marrow
	n		*kg.*	$\times 10^3$	%	$\times 10^6$	*g.%*	*g.%*	$\Delta n \times 10^4$		*mg.%*	*g.*	*g.%*	$\Delta n \times 10^4$		
B	0	1	2.41	10.8	76	6.35	13.6	6.13	120	19.6	29	—	—	—	—	—
		56	3.60	5.3	88	6.10	14.6	6.14	116	18.9	37	—	—	—	—	—
		110	3.80	13.5	86	6.05	11.2	6.71	121	18.0	39	14.900	0.452	7.9	17.5	0.238
D	0	1	2.33	10.5	88	6.05	13.6	5.62	103	18.3	37	—	—	—	—	—
		13	2.58	8.6	94	6.00	13.9	6.01	106	17.6	25	—	—	—	—	—
		56	3.13	11.8	84	6.50	14.3	5.87	109	18.6	41	11.425	0.831	15.2	18.3	0.243
E	0	—*	3.80	13.4	88	6.90	15.2	6.11	118	19.3	17	12.845	0.611	11.8	19.3	0.206
A	135†	1	2.10	8.9	92	6.20	14.6	5.52	108	19.6	29	—	—	—	—	—
		14	2.52	5.2	60	5.45	12.3	5.70	(112)‡	(19.6)	127	10.857	0.926	—	—	0.383
C	135†	1	2.43	10.4	80	6.00	13.1	5.63	104	18.5	29	—	—	—	—	—
		25	2.81	4.1	74	6.00	14.5	4.47	93	20.6	37	10.746	1.150	20.0	17.4	0.394

* This rabbit was an extra animal that had been used in the laboratory for some time for normal blood studies.

† Rabbits A and C were irradiated with 135 n of neutrons on the 13th day of observation.

‡ Corrected value—See *Effect of Neutron Irradiation on Plasma and Serum Composition, p. 100.*

the serum and plasma were diluted, immediately after removal of the cellular constituents, with three parts of buffer solution, then dialyzed against two changes of the same buffer. The nitrogen determinations are summarized in Table II and the electrophoretic data in Tables III, IV, V, and VI. The electrophoretic diagrams for a representative plasma and serum are given at the top of Fig. 2.

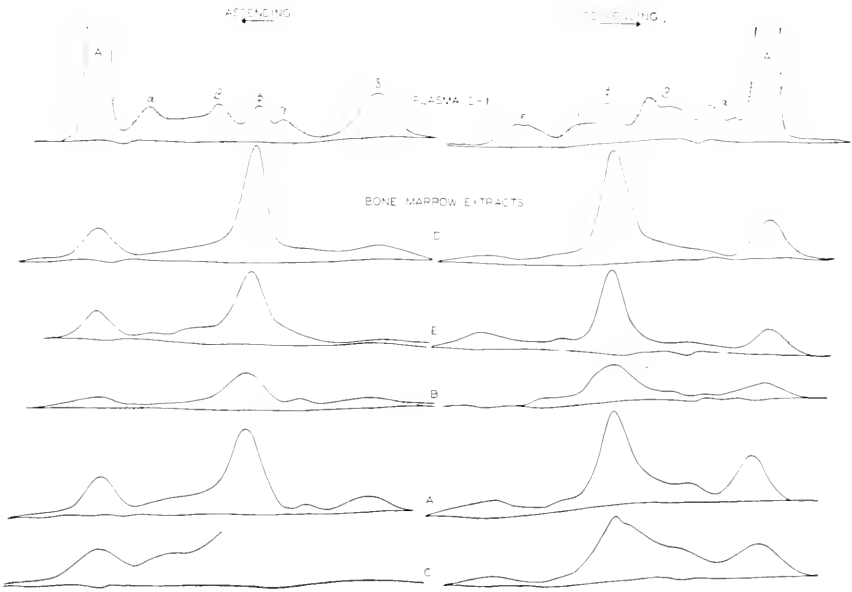


FIG. 2. Tracings of Svensson diagrams obtained by the electrophoresis of rabbit plasma, rabbit serum, and rabbit bone marrow extracts in 0.1 N sodium veronal-veronal buffer solution of pH 8.6. Plasma D-1: 95 minutes at 20 ma (114 coulombs); serum D-1 (dotted curve): 92 minutes at 20 ma (110.4 coulombs). Bone marrow extracts: 70 minutes at 27 ma (113.4 coulombs). Plasma and serum D-1 and bone marrow extracts D, E, and B: no irradiation. Bone marrow extract A: 1 day after irradiation with 135 n; solution clarified by centrifuging. Bone marrow extract C: 12 days after irradiation with 135 n; solution clarified by centrifuging. Bone marrow extract B, dotted curve: solution concentrated to half the volume two months later.

Bone Marrow. To obtain the bone marrow for study, the rabbits were exsanguinated from the heart, plasma and serum being prepared from this blood for both chemical and electrophoretic analysis. Immediately after death and as rapidly as possible, 10 long bones (2 each—femur, tibia, humerus, ulna and radius) were removed and freed of all adherent tissues. The cleaned bones were then frozen in solid carbon dioxide—a process that

TABLE III

Rabbit Plasma Composition on the First Day of Observation

	Concentrations (in terms of the undiluted samples) Computed from the Curve Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4													
	Descending Arm							Ascending Arm						
	Albumin	α	β	ϕ	γ	ϵ	Total	Albumin	α	β	ϕ	γ	δ	Total
Plasma A-1	60.6	10.8	15.0	5.4	6.7	8.8	107.3	58.4	9.5	15.6	4.4	6.7	14.1	108.7
Plasma C-1	53.4	11.0	18.0	5.2	6.4	8.6	102.6	54.3	9.6	17.2	5.9	5.8	13.3	106.1
Plasma D-1	59.5	9.4	14.6	4.3	6.4	7.5	101.7	57.1	7.8	13.7	5.4	6.7	14.5	105.2
Plasma B-1	55.9	10.7	26.6	10.9	7.9	9.4	121.4	52.5	8.9	23.0	11.1	7.5	15.6	118.6
Plasma E	64.4	9.5	15.3	3.2	16.0	9.6	118.0	63.0	6.8	14.2	3.5	15.4	15.2	118.0
Serum A-1	66.3	10.6	17.4	—	6.7	9.1	110.1	62.0	9.2	17.6	—	6.8	14.4	110.0
Serum C-1	65.5	12.4	21.6	—	7.8	9.9	117.2	63.9	11.2	21.0	—	7.4	15.1	118.6
Serum D-1	69.5	8.2	16.9	—	8.6	8.6	111.8	62.7	9.8	15.8	—	8.5	15.0	111.8
Serum B-1	57.3	11.3	27.8	—	7.9	8.5	112.8	60.8	10.2	27.6	—	8.8	15.3	122.7
Serum E	79.4	7.7	18.8	—	15.3	10.8	132.0	79.7	6.6	16.9	—	15.6	17.1	135.9

TABLE IV

Plasma and Serum Composition of Successive Blood Samples from Non-Irradiated Rabbits

	Day of Observation	Concentrations (in terms of the undiluted samples) Computed from the Curve Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4													
		Descending Arm							Ascending Arm						
		Albumin	α	β	ϕ	γ	ϵ	Total	Albumin	α	β	ϕ	γ	δ	Total
Plasma D-1	1	59.5	9.4	14.6	4.3	6.4	7.5	101.7	57.1	7.8	13.7	5.4	6.7	14.5	105.2
Plasma D-2	12	61.0	8.8	13.2	5.9	6.8	8.0	103.7	63.6	6.1	14.0	4.6	7.2	13.4	109.2
Plasma D-3	56	56.8	8.1	17.8	9.6	7.2	7.5	107.0	57.5	6.6	19.2	6.9	8.5	13.5	112.2
Serum D-1	1	69.5	8.2	16.9	—	8.6	8.6	111.8	62.7	9.8	15.8	—	8.5	15.0	111.8
Serum D-2	12	69.3	12.2	19.0	—	7.9	9.9	118.3	68.3	9.1	18.9	—	7.4	14.6	118.3
Serum D-3	56	74.0	9.2	24.2	—	11.3	9.3	128.0	70.4	8.5	22.6	—	9.7	16.8	128.0
Plasma B-1	1	55.9	10.7	26.6	10.9	7.9	9.4	121.4	52.5	8.9	23.0	11.1	7.5	15.6	118.6
Plasma B-2	56	No analysis.													
Plasma B-3	110	48.3	9.2	24.0	8.2	23.1	9.7	122.5	45.8	9.2	22.6	8.7	22.2	11.5	120.0
Serum B-1	1	57.3	11.3	27.8	—	7.9	8.5	112.8	60.8	10.2	27.6	—	8.8	15.3	122.7
Serum B-2	56	64.4	6.3	19.3	—	17.4	7.7	115.2	63.9	7.2	15.7	—	16.9	13.7	117.4
Serum B-3	110	50.9	10.8	25.8	—	24.1	10.3	121.9	—	—	—	—	—	—	—

TABLE V

Composition of Plasma and Serum from Irradiated Rabbits

	Day of Observation	Concentrations (in terms of the undiluted samples) Computed from the Curve Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^5													
		Descending Arm							Ascending Arm						
		Albumin	α	β	φ	γ	ε	Total	Albumin	α	β	φ	γ	δ	Total
Rabbit A															
Plasma A-1	1	60.6	10.8	15.0	5.1	6.7	8.8	107.3	58.4	9.5	15.6	4.1	6.7	14.1	108.7
	13	135 n administered.													
Plasma A-2	14	88.3	22.5	27.6	9.4	10.6	12.2	170.7	78.6	19.2	25.3	8.9	10.5	25.5	168.0
corrected*	14	57.8	14.7	18.1	6.2	6.9	8.0	111.7	52.2	12.8	16.8	5.9	7.0	17.0	111.7
Serum A-1	1	66.3	10.6	17.1	—	6.7	9.1	110.1	62.0	9.2	17.6	—	6.8	14.4	110.0
	13	135 n administered.													
Serum A-2	14	83.3	19.3	22.7	—	9.0	6.8	111.1	82.8	18.3	23.0	—	9.4	17.6	151.1
corrected*	14	65.9	15.3	18.0	—	7.1	5.4	111.7	61.3	13.5	17.0	—	6.9	13.0	111.7
Rabbit C															
Plasma C-1	1	53.4	11.0	18.0	5.2	6.4	8.6	102.6	54.3	9.6	17.2	5.9	5.8	13.3	106.1
	13	135 n administered.													
Plasma C-2	25	52.5	10.1	13.3	3.4	6.4	7.2	92.9	52.0	9.3	12.6	3.5	6.5	10.1	94.0
Serum C-1	1	65.5	12.1	21.6	—	7.8	9.9	117.2	63.9	11.2	21.0	—	7.4	15.1	118.6
	13	135 n administered.													
Serum C-2	25	57.1	8.8	14.7	—	6.5	7.9	95.0	55.6	9.0	13.2	—	6.0	10.4	94.2

* For correction factor see *Effect of Neutron Irradiation on Plasma and Serum Composition, p. 100.*

TABLE VI

Effect of Sonic Treatment on Rabbit Plasma

	Dilution during Sonic Treatment	Period of Sonic Treatment	Concentrations (in terms of the undiluted plasmas) Computed from the Curve Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments $\times 10^3$													
			Descending Arm							Ascending Arm						
			Albumin	α	β	ϕ	γ	ϵ	Total	Albumin	α	β	ϕ	γ	δ	Total
B-3		Untreated	48.3	9.2	24.0	8.2	23.1	9.7	122.5	45.7	9.2	22.6	8.7	22.2	11.6	120.0
B-3	1 → 4	30 min.	48.0	9.0	27.5	5.3	22.2	9.8	121.8	47.8	7.5	26.9	4.9	22.8	16.6	126.5
B-3	Undiluted	30 min.	52.5	8.3	29.3	5.8	23.6	9.0	128.5	49.0	9.2	26.1	5.3	25.5	11.6	129.6
C-2		Untreated	52.5	10.1	13.3	3.4	6.4	7.2	92.9	52.0	9.3	12.6	3.5	6.5	10.4	94.0
C-2	1 → 4	30 min.	51.3	7.7	13.5	3.3	6.1	6.0	87.9	50.8	8.0	13.3	3.0	5.0	9.6	89.7
E		Untreated	64.1	9.5	15.3	3.2	16.0	9.6	118.0	63.0	6.8	14.2	3.5	15.4	15.2	118.0
E	1 → 4	1 hour	66.8	7.4	15.6	3.9	15.8	9.4	118.9	66.0	6.9	15.6	3.0	14.1	13.4	119.0
D-3		Untreated	56.8	8.1	17.8	9.6	7.2	7.5	107.0	57.4	6.6	19.2	6.9	8.5	13.5	112.2
D-3	1 → 4	1 hour	63.2	7.7	25.6		9.5	11.9	118.2	61.3	8.1	25.4		10.7	16.5	122.0

was found in preliminary work to facilitate the removal of the bone marrow and its subsequent extraction.

The bones were split longitudinally and the marrow removed with forceps to a tared weighing bottle. After re-weighing, the bottle was kept in an ice bath while portions of the marrow were removed for homogenization.

The marrow was homogenized in phosphate-buffered normal saline (pH 7.3) containing 0.5 per cent lithium oxalate. Homogenization was brought about by a 5-minute exposure of 2 g. portions of marrow in 6 ml. of the above buffer to intense sound waves of 10.5 kc. produced by a Pierce magnetostriction oscillator comparable to that described by Chambers and Flosdorf (6). After each portion had been homogenized, it was removed to a centrifuge tube kept in an ice bath. The process was repeated until all of the bone marrow had been homogenized. The homogenate was kept overnight in the ice bath and then centrifuged. The fat layer was then removed and the aqueous extract filtered through sintered glass filters. A portion of the filtrate was analyzed for total and non-protein nitrogen and the remainder was dialyzed against four changes of buffer solution in preparation for electrophoresis. The chemical data on the bone marrow extracts are summarized in Table II. The fact that approximately twice the amount of protein was extracted from the marrow of the irradiated animals as from the non-irradiated group is of doubtful significance because of the small number of animals studied. The results of the electrophoretic experiments are given in Table VII, and the diagrams themselves are presented in Fig. 2 and Fig. 3.

A total of 30 to 40 minutes treatment in the magnetostriction oscillator was required for total homogenization of the marrow although each fraction required only five minutes. In order to determine whether or not any protein changes were produced by exposure to intense sound waves, plasma samples were similarly exposed for 30 to 40 minutes for rabbits B and C and for 60 minutes for rabbits D and E.

Pathology. On autopsy the non-irradiated rabbits, B, D, and E, showed no gross or microscopic pathological change. Of the two irradiated animals, one, rabbit C which was sacrificed 12 days after irradiation, was normal except for atrophy of the testes. However, the second irradiated animal, rabbit A, was found in a comatose state only 20 hours after irradiation. The plasma obtained from this animal had a milky opalescent appearance which could not be readily cleared up, and in addition gave a non-protein nitrogen value of 127 mg. per cent which was approximately three times the value found in the other four rabbits. On autopsy, this animal showed grossly a marked diarrhea and a spleen blue-grey in color. Microscopically the germinal centers of the lymphoid follicles of the small

TABLE VII

Composition of Bone Marrow Extracts from Non-irradiated and Irradiated Rabbits

Extract	Dose	Days after Irradiation	Concentrations Computed from Areas of Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4													
			Descending Arm							Ascending Arm						
			Albumin	α	β	ϕ	γ	ϵ	Total	Albumin	α	β	ϕ	γ	δ	Total
	n															
D			2.7 20%	0.6 4%	1.2 9%	7.9 58%	1.3 9%	1.3	15.0	2.7 20%	1.9 14%		7.7 56%	1.1 10%	1.7	15.4
E			2.1 21%	1.2 12%		5.8 58%	0.9 9%	1.8	11.8	2.1 20%	0.9 9%	0.8 8%	5.7 54%	1.0 9%	1.3	11.8
B			1.5 20%	1.0 13%		4.0 55%	0.9 12%		7.1	1.6 20%	1.1 14%		4.1 53%	1.0 13%	0.8	8.6
Remainder of B concentrated 2–1 two months later			2.8 20%	2.3 16%		7.1 50%	2.1 14%	1.6	15.9	2.9 21%	0.6 4%	1.6 12%	6.7 49%	1.9 14%	1.7	15.4
A Extract turbid	135	1	—	1.9		9.6	0.5	1.3	—	—	—	—	—	—	—	—
A Clarified	135	1	3.4 25%	1.1 8%	1.3 10%	7.0 50%	1.0 7%	1.5	15.3	3.1 23%	1.3 9%	1.2 9%	7.3 51%	0.6 5%	2.1	15.6
C Extract turbid	135	12	5.0 20%	3.7 22%		8.1 49%		1.8	18.9	6.0 32%	2.5 13%		10.3 55%		2.2	21.0
C Clarified	135	12	3.5 25%	2.5 17%	1.9 14%	5.6 40%	0.6 4%	1.1	15.2	3.3	2.3	2.1	—	—	—	—

intestine were necrotic although the epithelium of the mucous membrane was normal. The spleen showed marked atrophy of the malpighian corpuscles with abundant blood pigment in the monocytes. In general, a diagnosis of necrosis and atrophy of lymphoid tissue was made. However, it is difficult to ascribe these findings to the irradiation given the previous day. Although atrophy of lymphoid tissue normally follows neutron irradiation, it is not so pronounced one day after irradiation. Apparently,

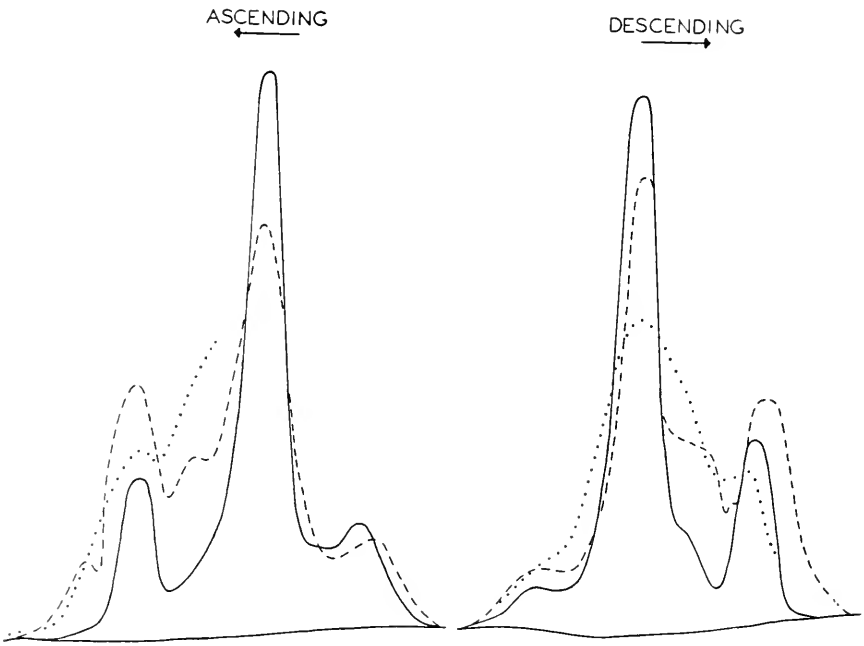


FIG. 3. Tracings of Longsworth diagrams obtained by electrophoresis of rabbit bone marrow extracts for 30 minutes at 27 ma (48.6 coulombs) in 0.1 N sodium veronal-veronal buffer solution of pH 8.6. Solid line: bone marrow extract D, no irradiation. Dashed line: bone marrow extract C, 12 days after irradiation with 135 n. Dotted line: bone marrow extract C, clarified by centrifuging.

this animal was ill prior to irradiation although no indications thereof were given by any of the data obtained prior to irradiation.

ELECTROPHORESIS EXPERIMENTS

All electrophoresis experiments were carried out in the Tiselius apparatus with the single-section cell of 11 ml. capacity, using a 0.1 N sodium veronal-veronal buffer solution of pH 8.6. For the plasma and serum solutions the current was kept at 20 ma, and for the bone marrow extracts at 27 ma. Photographs were taken by both the Longsworth (7) and Svensson (8)

methods. The bone marrow extracts, all of which were pigmented with hemoglobin, were photographed with three different exposures to insure clear pictures of all parts of the electrophoresis patterns. Area measurements were made on enlarged tracings of the Svensson diagrams. The protein concentrations computed from these areas, reported in Tables III, IV, V, and VI for the plasmas and sera, and in Table VII for the bone marrow extracts, are all expressed as refractive increments, since the exact specific refractive increments required to convert these values to grams per 100 ml. are not known. Each value represents the average from four different diagrams.

Electrophoretic analyses were made of plasma and serum from each blood sample in the expectation that the difference between the two analyses would yield the fibrinogen content with greater precision than is possible from analysis of the plasma alone. It was found, however, that the total protein content was as a rule greater for the serum than for the plasma, in spite of the absence from the serum of the clot-forming elements. Nevertheless, it was possible to superpose the plasma and serum patterns, as shown in the diagrams at the top of Fig. 2, and from the area between to determine the fibrinogen content with less error than from the plasma pattern alone. It can be seen from this diagram that part of the area which is assigned to fibrinogen by the usual method of dropping ordinates from the minima on either side of the fibrinogen peak belongs instead to the γ globulin.

Of the four animals initially selected for these experiments, three, A, C, and D, gave consistent plasma and serum analyses, as shown in Table III. The fourth, B, showed higher concentrations of β globulin and fibrinogen; and E, a fifth rabbit acquired later, showed a higher concentration of γ globulin. Rabbits A and C were therefore selected for neutron irradiation, and rabbits B, D, and E served as controls without irradiation.

Plasma and Serum Composition of Successive Blood Samples from Non-Irradiated Rabbits. Two of the three non-irradiated rabbits were under observation for several weeks. One of the two, rabbit D, which on the first day of observation closely resembled rabbits A and C with respect to plasma composition, showed very little variation in plasma composition throughout the period of observation. There was no significant change on the twelfth day, and only a slight rise in β globulin on the 56th day (Table IV). The other, rabbit B, which on the first day of observation had a higher content of β globulin and fibrinogen than A, C, and D, showed fairly wide fluctuations. On the 56th day the α and β globulin concentrations were diminished, and the γ globulin concentration was almost doubled. On the 110th day the plasma showed almost complete restoration of the α and β globulins, a small increase in fibrinogen with respect to

the first day, a still further increase in γ globulin beyond that of the 56th day, and a small decrease in albumin.

Effect of Neutron Irradiation on Plasma and Serum Composition. In view of the close resemblance, with respect to plasma composition, among rabbits A, C, and D on the first day of observation, the constancy of plasma composition for rabbit D throughout the period of observation appeared to warrant the conclusion that probably no significant change occurred in the plasma of rabbits A and C during the twelve days prior to irradiation, and that any changes observed later were largely the result of the irradiation and its secondary effects.

Rabbit A died less than 24 hours after irradiation. The plasma and serum obtained just prior to death were opaque with suspended material of low density. To remove this, the plasma and serum were frozen in small centrifuge tubes, then allowed to melt; a protein-impoverished layer was thus formed near the surface of each. When the samples were then subjected to prolonged centrifuging in a multispeed attachment with dry ice cooling, the suspended matter rose into the protein-impoverished layer, and was removed. The plasma and serum, now much clearer, but also more concentrated, were then diluted and dialyzed as usual for electrophoresis. The plasma protein concentrations computed from the resulting electrophoresis diagrams were corrected for the concentrating effect of this procedure on the basis of the nitrogen analysis of the plasma before centrifugation (see Table II), and of the ratio, $\frac{\Delta n \times 10^4}{g.\%} = 19.6$, for the plasma from the same animal on the first day of observation. The serum protein concentrations were arbitrarily corrected to the same total protein content as the plasma. The only change shown by the electrophoresis diagrams as a result of the irradiation was a possible slight increase in α globulin.

Rabbit C, which was sacrificed twelve days after irradiation, showed a diminution in total plasma protein concentration of about 10 per cent (Table V), resulting almost entirely from the decreased concentrations of β globulin and fibrinogen. There was little change, therefore, in the plasma protein composition of either rabbit following irradiation.

In a more extended study of the plasma proteins of irradiated rabbits (9), a diminished γ globulin content was found to be a characteristic response. It appears that rabbit A was examined too soon after irradiation for this response to have occurred; and for rabbit C the 12-day interval between irradiation and examination was sufficiently long for a return to the initial value (cf. Table IV of Chapter 14).

Effect of Sonic Treatment on Plasma. Samples of plasma from the final bleedings of rabbits B, C, D, and E were subjected to sonic treatment for the same length of time as the corresponding bone marrows. The results,

listed in Table VI, show that sonic treatment caused no significant change in the proteins of plasmas C and E diluted with three parts of buffer solution, nor of plasma B, whether diluted or undiluted. Defibrination occurred during dialysis of the sonicized sample of plasma D: but since defibrination has occasionally been observed during dialysis of plasmas which have not been sonicized, it is believed that the defibrination was caused by the undetermined factor operating in these other instances, rather than by the sonic treatment.

Electrophoretic Analysis of Bone Marrow Proteins. The bone marrow extracts from the three non-irradiated rabbits, B, D, and E, gave the electrophoresis diagrams shown at the middle of Fig. 2. By comparing these with the plasma diagram at the top of the figure, it can be seen that the bone marrow constituents had approximately the same mobilities as the plasma proteins, but were present in different proportions than in plasma. The composition of the three extracts was quite uniform (Table VII): each contained about 25 per cent of albumin, 15 per cent of α and β globulin, 55 per cent of fibrinogen, and 10 per cent of γ globulin. In view of the high albumin content of rabbit plasma, the comparatively low albumin content of the bone marrow extracts is an indication that the bone marrow proteins were, at most, only slightly contaminated by plasma proteins from residual blood in the marrow.

A second analysis was made on bone marrow extract B after two months in the refrigerator; the remaining solution was reduced to half its original volume by pressure dialysis. The resulting electrophoresis diagram is given by the dotted curve for extract B in Fig. 2. The good agreement shown by the two experiments (see also Table VII) is evidence for the stability of the extract.

The bone marrow extracts of the two irradiated rabbits, A and C, were turbid. Part of each was clarified by centrifuging for an hour at about 10,000 r.p.m. in a multispeed attachment with dry ice cooling. Electrophoresis was carried out on both the clear and the turbid portions of each.

The clarified portions of the extracts gave the electrophoresis diagrams reproduced at the bottom of Fig. 2. For rabbit A, obtained one day after irradiation, there was no significant difference from the non-irradiated rabbits. For rabbit C, sacrificed 12 days after irradiation, the percentages of albumin, α globulin and β globulin were higher, and the percentages of fibrinogen and γ globulin were lower (Table VII). The turbid portion of extract C showed the same difference with respect to the extracts from the non-irradiated rabbits. This is demonstrated in Fig. 3, in which the dashed lines represent the diagram for rabbit C, and the solid line the diagram for rabbit D, which was not irradiated. The dotted line in this figure represents extract C clarified. Centrifugation resulted in a reduction

in the total protein content for both extract A and extract C, and a broadening and overlapping of the fibrinogen and γ globulin boundaries for extract C.

SUMMARY

Preliminary electrophoresis studies have been made on the plasma, serum, and sonicized bone marrow extracts of five rabbits, two of which were irradiated with a single dose of 135 n.

An anomalous relationship was found between the protein concentrations of the plasmas and the corresponding sera.

When examined by electrophoresis, samples of plasma subjected to sonic treatment similar to that given the corresponding bone marrow showed no significant differences from the untreated samples.

Slight differences were observed between the data for irradiated and non-irradiated rabbits, but in view of the paucity of experimental animals, these differences must be corroborated by further evidence before definite conclusions can be drawn.

REFERENCES

(1) Leitch, J. L., Chapter 4.
(2) Müller, P. Th., *Hofmeistr. Beiträge*, **6,** 454 (1905).
(3) Keilhack, H., *Arch. exptl. Path. Pharmakol.*, **180,** 440 (1936).
(4) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(5) Tiselius, A., *Trans. Faraday Soc.*, **33,** 524 (1937); *Biochem. J.*, **31,** 1464 (1937).
(6) Chambers, L. A., and Flosdorf, E. W., *Proc. Soc. Exptl. Biol. Med.*, **34,** 631 (1936).
(7) Longsworth, L. G., *J. Am. Chem. Soc.*, **61,** 529 (1939).
(8) Svensson, H., *Kolloid-Z.*, **87,** 181 (1939).
(9) Sanigar, E. B., Miller, G. L., and Maddox, M. N., Chapter 14.

CHAPTER 13

ELECTROPHORESIS OF BLOOD PLASMA OF NEUTRON-IRRADIATED CHICKENS

BY LAURA E. KREJCI AND LUCILE SWEENY

A preliminary study was made of the effect of a single sublethal dose of neutrons on the blood plasma of young chickens. The changes in plasma protein composition were followed by means of the Tiselius electrophoresis technique (1), and observations were continued for four months subsequent to irradiation.

MATERIALS AND METHODS

Barred Rock chickens were used for the investigation. They were obtained from the University of Delaware Agriculture Experiment Station and were maintained on a diet of Purina Chick Growena and water *ad libitum*. Three males, two at the age of 82 days, the third at the age of 103 days, were given a single neutron dose of 169 n (2). Two males, 104 days old, were used as controls without irradiation. The irradiated chickens were bled for plasma and serum samples before irradiation and at intervals of increasing length for four months afterwards; the control chickens were bled at approximately the same intervals for a period of two months. At the termination of the experiment all five were sacrificed and autopsied.

Before each bleeding the chickens were deprived of food for 24 hours to insure plasma and serum free from suspended fats. Ten ml. of blood were drawn by cardiac puncture (3); 5 ml. were placed in a tube containing 0.5 mg. dry lithium oxalate as anticoagulant for use as plasma, and the rest was converted to serum. Immediately after removal from the cells and clot, the plasma and serum were diluted with three parts of a sodium veronal-veronal buffer solution of pH 8.6 then dialyzed against two changes of the same buffer solution in preparation for electrophoresis.

A current of 27 ma was used during electrophoresis, and photographs were taken by the Longsworth (4) and Svensson (5) methods for four different periods of migration. Protein concentrations were computed from area measurements made on enlarged tracings of the four Svensson diagrams. These concentrations have been reported in terms of the refractive increments, because the specific refractive increments required to convert the values to grams per 100 ml. are not accurately known. The electrophoresis diagrams were divided into the alpha, beta, and gamma globulin fractions in the manner suggested by Sanders, Huddleson and

Schaible (6). A different division has been suggested by Deutsch and Goodloe (7).

EXPERIMENTAL RESULTS

In the electrophoresis diagrams of chicken plasma, the fibrinogen does not form the clear-cut boundary characteristic of fibrinogen in human plasma, but lies above the advance portion of the gamma globulin boundary, as shown by the superposed plasma and serum diagrams reproduced

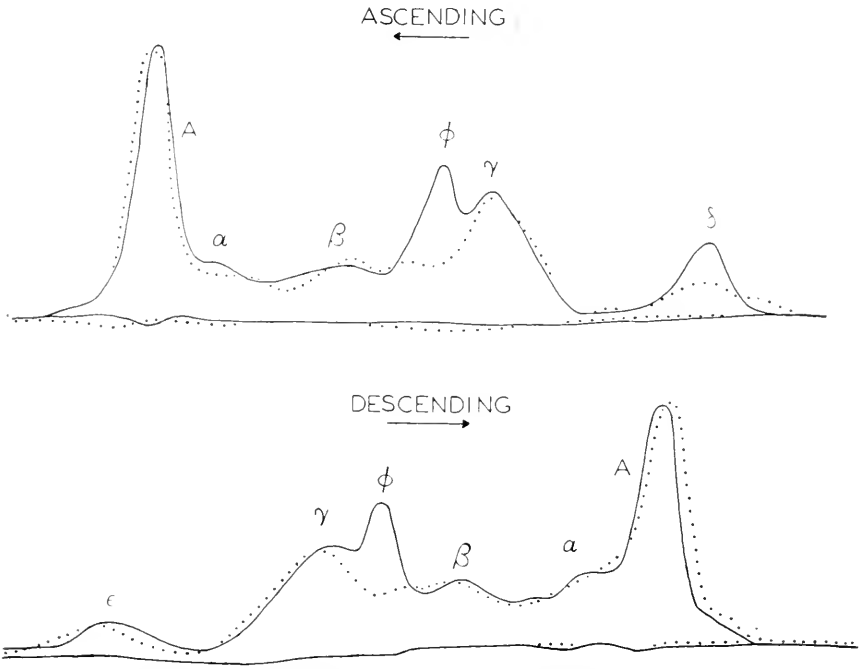


FIG. 1. Tracings of Svensson diagrams for plasma 27-0 and serum 27-0 before irradiation. Electrophoresis in 0.1 N sodium veronal-veronal buffer solution of pH 8.6 for 70 minutes at 27 ma.

in Fig. 1. Determinations of fibrinogen concentration made by dropping an ordinate at the minimum on each side of the fibrinogen maximum, therefore, represent an overestimate. The fibrinogen content might be expected to be given with greatest precision by the difference in protein concentration between the plasma and the corresponding serum.

In the present investigation parallel plasma and serum analyses were made on 41 pairs of solutions. The results are listed in Tables I, II, and III. All but five of the sera (the five exceptions are marked with a dagger

in Tables II and III) had a protein content higher than that of the corresponding plasma minus the fibrinogen; and 23 (marked with an asterisk in Tables II and III) had a protein concentration equal to or greater than that of the corresponding plasma. Of nine rabbit sera studied in another investigation (8), eight were more concentrated than the corresponding plasma, and the ninth had a concentration greater than that of the corresponding plasma minus the fibrinogen. On the basis of preliminary

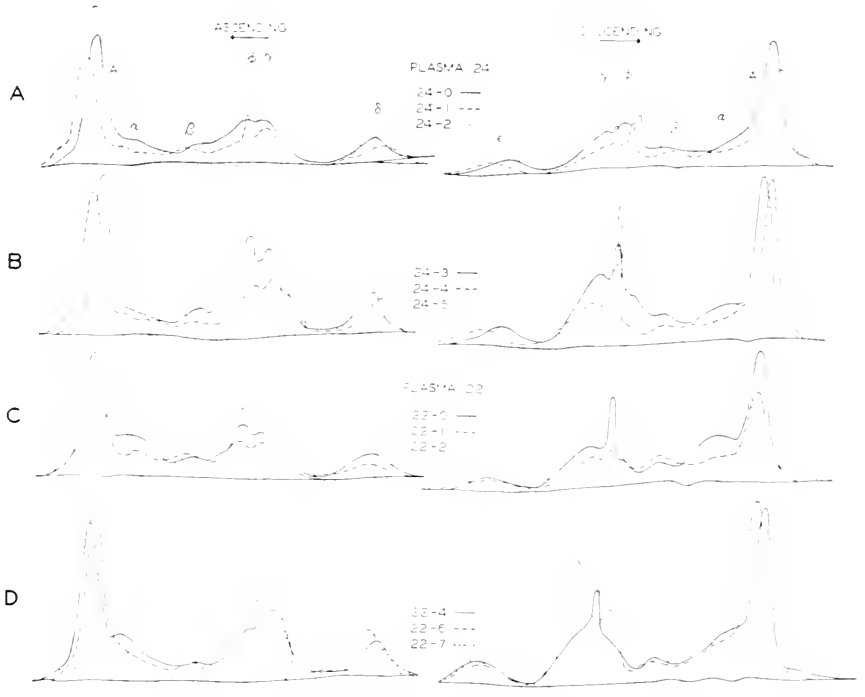


FIG. 2. Tracings of Svensson diagrams obtained by electrophoresis of chicken plasmas in 0.1 N sodium veronal-veronal buffer solution of pH 8.6 for 70 minutes at 27 ma. See Table I.

experiments by Dr. James L. Leitch of this Laboratory, plasma samples prepared from the same lot of chicken blood, when analyzed by the micromethod of Kjeldahl, appear to be not only lower, but also more uniform, in concentration than samples of serum. The results of the present investigation will therefore be discussed in terms of the plasma composition.

In spite of the elevated protein levels of most of the sera, it was possible to superpose the plasma and serum diagrams with the beta globulin and gamma globulin peaks matching, and, by measuring the area between,

determine the fibrinogen content with less error than from the plasma diagrams alone. The uncertainty involved, however, introduced the possibility of error in the concentrations of both the gamma globulin and the fibrinogen. To eliminate these compensating errors, the fibrinogen and gamma globulin concentrations were plotted as the sum in the graphical presentation of the data. Because of the poor resolution of the alpha and beta globulin boundaries, the concentrations of alpha globulin and beta globulin were also plotted as the sum.

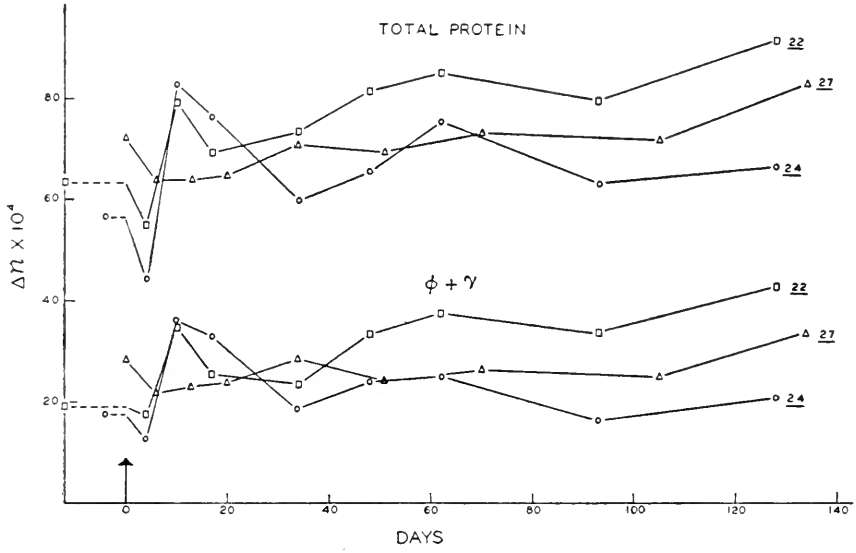


FIG. 3. Changes in the composition of chicken plasma following neutron irradiation. Ordinates: concentrations expressed in refractive increments $\times 10^4$. Abscissae: days after irradiation. Chicken 22: □; chicken 24: ○; chicken 27: △. Upper graphs: total protein concentration. Lower graphs: concentration of fibrinogen plus gamma globulin.

Irradiated Chickens. Representative electrophoresis diagrams for chickens 24 and 22, both irradiated at the age of 82 days, are shown respectively in A and B, and in C and D, of Fig. 2. The corresponding concentration changes are listed in Table I and presented graphically in Figs. 3 and 4.

From the variations in the diagrams of Fig. 2, and from the graphical presentation of the corresponding changes in total protein concentration at the top of Fig. 3, it can be seen that for chickens 22 and 24 there was a drop in total protein concentration during the four days immediately following irradiation. This drop was overcompensated during the next six days by an abrupt rise in protein concentration, greater for chicken 24, of

lower initial protein concentration, than for chicken 22, to a point high above the original value. This in turn was followed by a gradual drop, greater and more prolonged for chicken 24 than for chicken 22, to a point somewhat above the initial value. Instead of remaining at these levels, however, the protein concentrations for both chickens continued to fluctuate, but with diminishing amplitude and frequency, throughout the period of observation.

The graphs for chickens 22 and 24 in Figs. 3 and 4 show that the drop in protein concentration during the four days following irradiation was the resultant of diminished concentrations of each of the three groups of plasma constituents. For the concentrations of albumin and of alpha globulin plus beta globulin, however, the subsequent rise was about equal to the

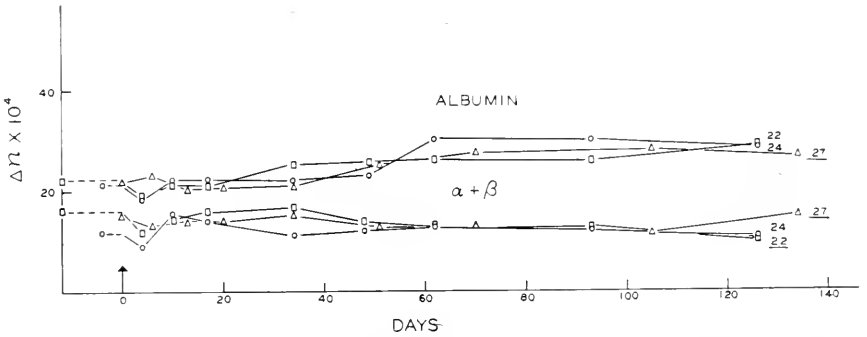


FIG. 4. Changes in the composition of chicken plasma following neutron irradiation. Ordinates: concentrations expressed in refractive increments $\times 10^4$. Abscissae: days after irradiation. Chicken 22: □; chicken 24: ○; chicken 27: △; Upper graphs: concentration of albumin. Lower graphs: concentrations of alpha globulin plus beta globulin.

initial drop, and later changes were small. On the other hand, as shown in Fig. 3, the fluctuations of the total protein content were paralleled, and to a large extent determined, by the fluctuations in the concentrations of fibrinogen plus gamma globulin.

During the three weeks immediately following irradiation, the plasma samples for chicken 27 were obtained at times intermediate between those for chickens 22 and 24 in order to follow more closely the pattern of change. The high variability of the protein concentrations, however, necessitates separate consideration of each chicken.

Like chickens 22 and 24, chicken 27 experienced a drop in protein concentration following irradiation; but no overcompensating increase was detected. Since the data for chickens 22 and 24 strongly indicate a minimum concentration at about four days, and a maximum concentration at

about ten days, following irradiation, it is possible that the plasmas for chicken 27 were not obtained at the proper times to demonstrate the rise in concentration.

Non-Irradiated Chickens. The changes in plasma composition for the non-irradiated chickens, 69 and 80, are listed in Table III and presented

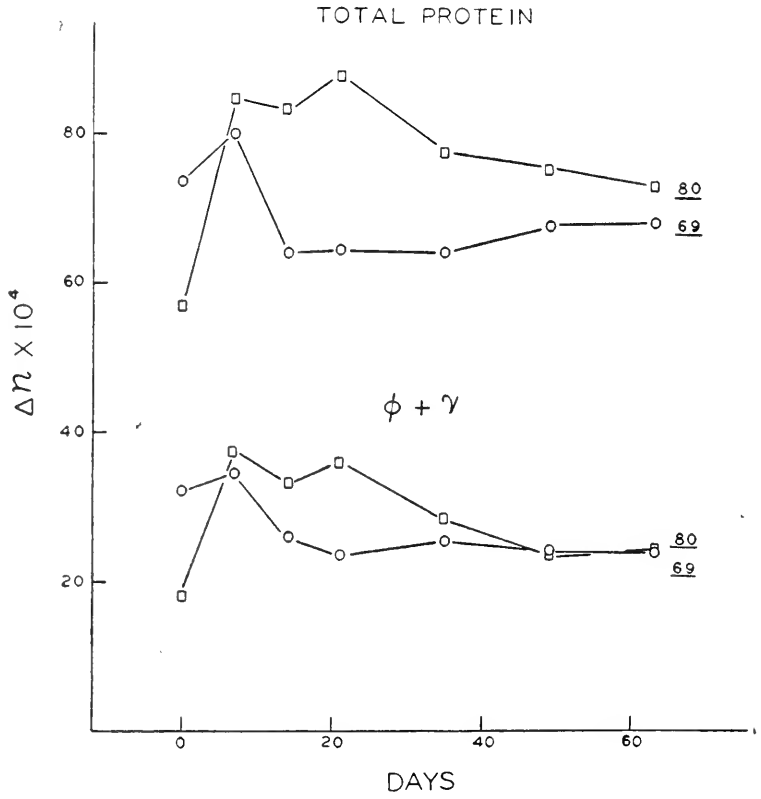


FIG. 5. Composition of successive samples of plasma from non-irradiated chickens. Ordinates: concentrations expressed in refractive increments $\times 10^4$. Abscissae: days after irradiation. Chicken 69: ○; chicken 80: □. Upper graphs: total protein concentration. Lower graphs: concentration of fibrinogen plus gamma globulin.

graphically in Figs. 5 and 6. For both chickens there was an increase of protein concentration during the week following the taking of the first blood samples; the increase was greater for chicken 80, of lower initial protein concentration, than for chicken 69.

Like the irradiated chickens, the non-irradiated chickens showed little variation in the concentrations of albumin and of alpha globulin plus beta

globulin. The variations in total concentration were largely a reflection of variations in the concentrations of fibrinogen plus gamma globulin.

GROSS PATHOLOGY

Only chicken 22 showed gross pathological changes of significance. The heart was dilated, and the walls were thin. This hypertrophy may be attributed to anemia resulting from the repeated bleedings. An adhesion between the pericardial sac and the heart may likewise have been due to the bleeding. The spleen appeared to be slightly reduced in size. The bone marrow was very red. All other tissues appeared normal.

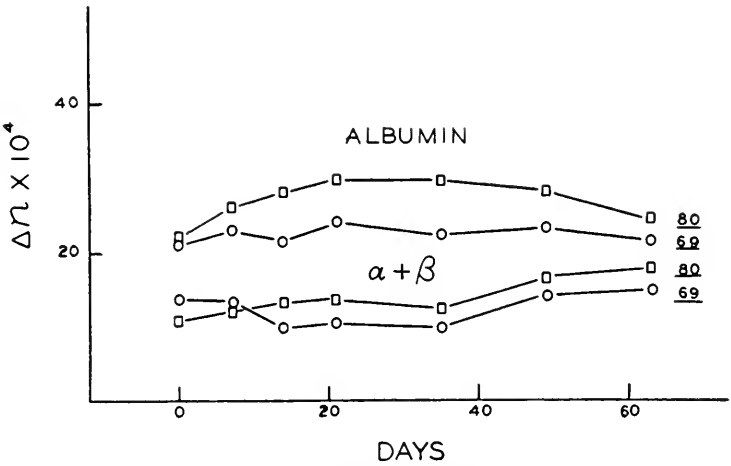


FIG. 6. Composition of successive samples of plasma from non-irradiated chickens. Ordinates: concentrations expressed in refractive increments $\times 10^4$. Abscissae: days after irradiation. Chicken 69: ○; chicken 80: □. Upper graphs: concentration of albumin. Lower graphs: concentration of alpha globulin plus beta globulin.

DISCUSSION

The lymphocytes are a storehouse of readily available gamma globulin which can be released either through dissolution of the lymphocytes by adrenal cortical stimulation, or through direct destruction of the lymphocytic elements without hormone mediation (9). Neutron irradiation inhibits production of lymphocytes, and the lymphocytes already in circulation are either destroyed or gradually lost (10). In the chicken production of lymphocytes was found in this Laboratory to be resumed on the third day following neutron irradiation.

Because of the small number of animals examined in this study, definite conclusions are hardly warranted. It may be pointed out, however, that

the abrupt increase in gamma globulin levels between the fourth and tenth days following irradiation is coincident with resumption of lymphocyte production. It may also be pointed out that the subsequent parallel fluctuations in gamma globulin concentration and total plasma protein concentration may reasonably be the result of fluctuations in hormonal stimulation of lymphocyte dissolution, for plasma protein depletion is in effect similar to hemorrhage, one of the known stimuli of the pituitary-adrenal cortical mechanism.

SUMMARY

Parallel electrophoretic analyses have been made on the plasma and serum of three chickens given a single dose of 169 n, and of two non-irradiated chickens.

An anomalous relationship was found between the protein concentrations of the plasmas and the corresponding sera.

The single dose of 169 n was followed on the fourth day by a reduction in concentration of all the plasma proteins, and during the four subsequent months by parallel fluctuations in the concentrations of gamma globulin and total plasma protein.

REFERENCES

(1) Tiselius, A., *Trans. Faraday Soc.*, **33**, 524 (1937).
(2) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(3) Genest, P., *J. Vet. Med. Assoc.*, **108**, 239 (1946).
(4) Longsworth, L. G., *J. Am. Chem. Soc.*, **61**, 529 (1939).
(5) Svensson, H., *Kolloid-Z.*, **87**, 181 (1939).
(6) Sanders, E., Huddleson, I. F., and Schaible, P. J., *J. Biol. Chem.*, **155**, 469 (1944).
(7) Deutsch, H. F., and Goodloe, M. B., *J. Biol. Chem.*, **161**, 1 (1945).
(8) Krejci, L. E., Leitch, J. L., and Sweeny, L., Chapter 12.
(9) White, A., and Dougherty, T. F., *Ann. N. Y. Acad. Sci.*, **46**, 859 (1946).
(10) Ross, M. H., and Ely, J. O., Chapter 18.

TABLE I

The Electrophoretic Analysis of Chicken Plasmas, Showing the Effects of Neutron Irradiation

Plasma	Days after Irradiation	Concentrations (in terms of the undiluted plasmas) Computed from the Curve Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4													
		Descending Arm							Ascending Arm						
		Albumin	α	β	φ	γ	ε	Total	Albumin	α	β	φ	γ	δ	Total
22-0	−12	23.0	13.7	3.4	4.5	14.3	4.6	63.6	21.9	11.9	3.6	4.1	15.1	6.6	63.2
22-1	4	15.6	7.6	6.5	3.6	11.3	5.4	52.0	18.8	5.5	7.3	3.8	13.1	4.8	53.3
22-2	10	21.4	11.2	5.0	4.3	29.9	7.5	79.3	21.3	8.6	5.0	4.4	31.4	8.2	78.9
22-3	17	21.2	9.7	8.5	4.4	18.0	5.3	67.1	21.2	7.8	5.9	5.2	23.3	7.8	71.2
22-4	34	25.4	14.0	3.9	3.4	19.0	6.9	72.6	25.3	11.6	3.9	3.8	21.9	7.5	74.0
22-5	48	25.9	6.2	9.6	4.5	28.3	6.9	81.4	25.9	6.2	6.0	4.2	29.6	9.4	81.3
22-6	62	26.1	9.5	4.1	4.7	31.5	7.2	83.1	26.3	7.8	3.5	5.3	33.7	9.8	86.4
22-7	93	25.5	9.1	5.1	6.9	26.5	6.4	79.5	26.0	6.8	4.6	7.5	26.8	7.8	79.5
22-8	126	29.2	5.8	6.1	7.6	35.2	6.7	90.6	28.9	4.5	5.0	7.3	35.6	10.6	91.9
24-0	−4	22.1	8.6	3.8	3.2	13.8	5.5	57.0	20.6	8.0	3.2	2.4	15.7	6.1	56.0
24-1	4	18.2	5.6	3.9	3.9	7.8	3.9	43.3	19.0	5.0	3.4	3.2	10.2	4.7	45.7
24-2	10	22.5	12.1	4.9	4.9	29.6	6.7	80.7	22.4	9.0	5.3	5.0	32.7	10.1	84.5
24-3	17	22.6	10.1	5.0	8.0	24.2	5.0	74.9	22.4	7.9	5.1	8.3	25.5	8.5	77.7
24-4	34	22.3	8.6	3.5	5.5	14.6	5.3	59.8	22.2	7.3	2.8	6.1	15.8	5.7	59.9
24-5	48	23.1	7.5	5.5	6.6	15.5	5.7	63.9	23.3	6.2	4.9	8.8	17.2	6.6	67.0
24-6	62	31.0	9.4	4.7	6.7	17.0	5.2	74.0	30.0	6.7	4.5	6.7	19.7	9.1	76.7
24-7	93	29.8	5.3	6.9	2.3	14.2	4.7	63.2	30.4	5.1	5.5	1.9	14.3	6.0	63.2
24-8	126	28.6	6.3	5.6	4.4	16.1	6.8	67.8	28.1	4.0	5.5	4.0	17.3	6.4	65.3
27-0	0	21.8	8.5	8.3	5.8	22.2	5.7	72.3	22.3	6.0	7.9	5.8	22.9	7.1	72.0
27-1	6	22.0	10.7	4.6	7.2	13.6	5.1	63.2	24.6	6.6	4.4	6.6	15.9	6.0	64.1
27-2	13	20.4	7.7	8.3	6.8	15.4	5.1	63.7	20.6	6.3	6.8	6.3	17.6	6.2	63.8
27-3	20	20.4	7.2	8.6	5.5	17.4	5.2	64.3	21.0	5.7	6.8	5.0	19.8	6.7	65.0
27-4	34	21.2	10.3	5.0	4.5	24.1	5.8	70.9	—	—	—	—	—	—	—
27-5	51	27.5	12.4		2.7	21.7	4.9	69.2	22.8	6.9	6.2	2.8	21.3	7.5	67.5
27-6	70	26.6	7.5	6.4	3.9	21.4	5.6	71.4	28.3	6.0	5.5	3.4	24.2	7.5	74.0
27-7	105	27.8	4.7	7.1	4.1	20.7	5.8	70.4	28.1	5.7	6.0	4.0	21.4	7.8	72.9
27-8	134	25.8	11.4	5.3	3.3	29.6	6.4	81.9	27.1	7.9	5.2	3.0	31.6	8.9	83.8

TABLE II

The Electrophoretic Analysis of Chicken Sera, Showing the Effects of Neutron Irradiation

Serum	Days after Irradiation	Concentrations (in terms of the undiluted sera) Computed from the Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4											
		Descending Arm						Ascending Arm					
		Albumin	α	β	γ	ϵ	Total	Albumin	α	β	γ	δ	Total
22-0*	−12	25.9	11.8	8.5	15.2	5.0	66.4	25.1	10.8	6.8	14.9	7.4	65.0
22-1*	4	21.2	9.4	6.3	12.2	5.5	54.6	21.8	7.0	6.8	13.3	5.1	54.0
22-2*	10	23.1	11.0	6.8	33.6	6.7	81.2	23.3	7.8	7.2	37.3	8.6	84.2
22-3*	17	22.5	11.1	7.3	27.1	7.0	75.0	25.2	5.7	5.2	34.1	8.3	78.5
22-4*	34	29.2	11.1	7.5	20.4	6.7	74.9	30.2	8.9	8.1	21.6	8.6	77.4
22-5	48	27.0	7.2	5.2	34.2	5.0	78.6	27.5	6.6	4.5	34.6	8.9	82.1
22-6*	62	29.9	11.9	5.2	40.5	7.3	94.8	30.6	6.8	4.3	38.9	11.2	91.8
22-7	93	28.6	8.5	5.7	29.3	5.5	77.6	26.1	7.3	6.0	29.5	7.2	76.1
22-8*	126	33.7	8.1	9.1	38.8	9.4	99.1	33.0	6.3	8.2	39.2	10.7	97.4
24-0*	−4	23.7	9.2	3.4	16.9	5.1	58.3	24.1	7.9	4.1	18.5	6.4	61.0
24-1*	4	19.0	6.8	6.7	9.1	4.5	46.1	18.8	6.6	5.3	8.9	3.8	43.4
24-2*	10	24.4	12.2	5.2	36.3	7.1	85.2	23.8	8.6	5.7	40.1	11.1	89.3
24-3	17	25.9	9.8	8.0	23.8	6.0	73.5	25.0	8.4	6.7	25.0	6.7	71.8
24-4	34	22.5	6.4	6.5	15.1	7.9	55.4	23.8	7.8	4.6	15.9	5.5	57.6
24-5*	48	28.1	8.1	10.2	15.7	6.2	68.3	28.1	6.7	8.8	17.1	6.9	67.6
24-6*	62	32.1	7.3	11.9	18.8	6.1	76.2	33.0	7.8	8.6	19.1	7.8	76.3
24-7	93	29.9	6.3	6.3	13.5	3.9	59.9	30.3	6.2	5.7	15.9	5.0	63.1
24-8†	126	29.8	5.1	6.1	16.2	5.9	63.1	29.9	4.6	4.9	15.7	5.8	60.9
27-0	0	24.1	9.3	8.4	24.1	5.7	71.6	25.3	6.0	8.5	24.1	6.0	69.9
27-1	6	26.4	10.2	5.6	13.4	4.6	60.2	28.7	5.8	6.6	14.1	5.6	60.8
27-2†	13	20.2	5.7	8.0	14.0	3.8	51.7	21.9	6.2	5.6	16.9	5.1	55.7
27-3	20	22.4	6.8	9.3	18.5	6.0	63.0	23.6	6.4	6.9	22.6	7.4	66.3
27-4*	34	27.5	7.2	9.2	24.2	6.1	74.2	27.3	5.6	6.4	26.3	5.3	70.9
27-5*	51	28.9	7.1	9.8	22.1	5.6	73.5	29.8	5.2	6.5	26.9	7.3	75.7
27-6*	76	32.6	8.1	6.3	27.4	6.0	80.4	33.7	5.2	6.4	27.0	8.4	80.7
27-7	105	30.3	4.6	6.7	21.6	5.5	68.7	29.7	4.5	5.8	23.2	6.3	69.5
27-8*	134	28.3	12.6	6.3	30.6	8.4	86.2	29.1	7.8	5.1	31.8	8.1	81.9

* Protein concentration of serum exceeds concentration of corresponding plasma.

† Protein concentration of serum is less than the concentration of the corresponding plasma minus the fibrinogen.

TABLE III

The Electrophoretic Analysis of the Plasmas and Sera of Non-Irradiated Chickens

Plasma	Day of Observation	Concentrations (in terms of the undiluted samples) Computed from the Areas of the Electrophoresis Diagrams and Expressed in Refractive Increments × 10^4													
		Descending Arm							Ascending Arm						
		Albumin	α	β	ϕ	γ	ϵ	Total	Albumin	α	β	ϕ	γ	δ	Total
69-1	0	19.7	7.3	6.8	6.1	26.8	7.2	73.9	22.1	6.0	7.5	6.7	24.4	6.8	73.5
69-2	7	22.6	7.4	7.7	7.9	25.6	7.0	78.2	23.6	4.9	7.3	7.2	28.6	10.5	82.1
69-3	14	21.9	5.8	6.0	4.7	20.4	5.5	64.3	21.3	4.4	4.3	5.0	21.2	7.3	63.5
69-4	21	24.3	3.7	7.5	3.9	19.5	5.2	64.1	23.8	3.4	6.4	3.5	21.0	6.6	64.7
69-5	35	23.1	5.8	5.2	4.7	20.6	6.9	66.3	21.6	4.1	3.9	5.1	20.4	6.6	61.7
69-6	49	24.3	9.4	4.9	5.0	18.6	5.8	68.0	22.3	9.0	4.7	5.7	19.3	6.2	67.2
69-7	63	20.3	12.6	4.4	4.0	21.8	6.5	69.6	22.7	8.7	4.1	4.0	20.5	6.4	66.4
80-1	0	21.9	4.7	6.8	4.1	13.8	4.9	56.2	21.9	3.8	6.7	3.4	15.2	6.7	57.7
80-2	7	27.4	3.9	9.8	7.6	28.9	8.5	86.1	25.7	3.9	6.8	6.0	32.2	8.8	83.4
80-3	14	28.4	5.8	7.9	4.6	27.4	7.7	81.8	27.7	4.1	8.8	5.3	29.4	9.1	84.4
80-4	21	29.8	5.8	9.0	6.1	29.0	8.3	88.0	29.6	3.8	8.8	5.6	31.4	8.3	87.5
80-5	35	29.3	7.4	6.3	5.7	21.6	7.0	77.3	29.8	4.0	7.2	6.2	22.4	8.3	77.9
80-6	49	27.2	13.0	5.3	5.0	18.4	6.2	75.1	28.8	9.1	5.3	5.3	19.7	7.3	75.5
80-7	63	23.8	15.3	4.3	3.6	19.6	6.3	72.9	24.8	10.9	4.8	3.5	22.2	7.3	73.5
Serum															
69-1	0	24.6	6.9	7.8	—	25.9	6.9	72.1	24.1	5.2	6.1	—	26.0	8.5	69.9
69-2†	7	23.0	7.4	7.4	—	26.9	7.3	72.0	23.1	4.6	7.8	—	26.6	7.4	69.5
69-3	14	22.8	6.0	6.2	—	20.4	6.6	62.0	18.7	7.1	6.6	—	22.0	5.2	59.6
69-4*	21	26.6	6.6	7.4	—	21.3	6.8	68.7	26.7	4.4	6.7	—	22.1	7.4	67.3
69-5†	35	24.7	4.1	4.6	—	20.2	5.1	58.7	24.3	6.1		—	22.0	5.6	58.0
69-6*	49	22.5	13.0	8.0	—	19.6	5.2	68.3	24.9	8.0	7.6	—	20.5	7.6	68.6
69-7	63	26.0	10.2	11.4	—	19.9	5.0	65.5	23.2	10.6	3.8	—	22.6	6.6	66.8
80-1*	0	28.9	5.2	9.4	—	18.2	6.9	68.6	27.4	5.3	8.6	—	19.7	7.9	68.9
80-2†	7	26.6	5.6	8.9	—	25.3	5.3	71.7	26.5	3.0	7.1	—	27.1	7.3	71.0
80-3	14	29.7	6.8	8.1	—	29.3	6.8	80.7	28.8	4.4	8.3	—	30.1	8.9	80.5
80-4*	21	34.1	6.2	10.9	—	32.6	7.4	90.9	33.3	5.0	8.4	—	34.0	9.5	90.2
80-5*	35	36.0	6.0	8.9	—	23.8	5.9	80.6	35.5	4.5	7.2	—	24.1	8.1	79.4
80-6*	49	30.4	10.4	9.1	—	19.2	8.9	78.0	31.4	8.1	8.4	—	20.9	10.5	79.3
80-7*	63	28.2	13.1	5.2	—	22.3	6.6	75.4	27.2	11.4	4.6	—	23.1	7.8	74.1

* Protein concentration of serum exceeds concentration of corresponding plasma.

† Protein concentration of serum is less than the concentration of the corresponding plasma minus the fibrinogen.

Chapter 14

ELECTROPHORESIS OF THE PLASMA OF RABBITS IRRADIATED WITH NEUTRONS

By EDWARD B. SANIGAR, GAIL L. MILLER, and MARY NORTON MADDOX

To obtain information concerning the mechanism of the action of neutron irradiation on living animals a study has been made, by means of the moving boundary electrophoretic method (1), of the changes in blood plasma composition during and after neutron irradiation of rabbits. Rabbits were chosen for the investigation so that sufficient blood could be withdrawn for each experiment without sacrificing or seriously harming the test animals.

Further, it appeared desirable to relate some relatively simple biological response, which could be observed along with the electrophoretic study of the plasma, to the size of the irradiation dose. A suitable response was the well-established decrease in white blood cell count which results from neutron irradiation (2, 3). Thus the relationships between amount of irradiation, plasma protein composition and white blood cell count were studied with two types of treatment, namely, various irradiation doses given over short periods of time and large amounts administered in small doses over long periods.

Changes in body weight and the development of outstanding physical abnormalities by the animals were also noted during the work. Autopsies were performed at the termination of certain of the experiments to ascertain whether any gross body changes had resulted from irradiation.

To determine whether neutron irradiation had a direct effect on the electrophoretic composition of rabbit plasma, aliquots of a sample of plasma from one rabbit were exposed *in vitro* to various large neutron doses and then tested for possible changes in electrophoretic pattern.

MATERIALS AND METHODS

Animals. Male, white New Zealand rabbits, 4½ months old and weighing approximately 6 pounds, were used. They were kept in galvanized wire cages and maintained on a diet of Purina Rabbit Chow and water.

Irradiation. The production of the neutrons and the method of irradiation have already been described by Enns *et al.* (4). The daily irradiation of 55 n given one series of rabbits in cage 4, was administered continuously in a single dose over a period of about 3½ hours. Daily irradiation of 5 or 10 n given other groups in cage 5, was intermittent, the total per day being administered over periods of 1–2 hours.

White Blood Cell Counts. Daily white cell counts were made by standard procedure on blood obtained from the marginal ear vein. The counts for most of the rabbits were made for 2–4 weeks prior to irradiation to establish the individual normal value.

Electrophoresis Measurements. Blood for electrophoresis was obtained by heart puncture from animals from which food had been withheld overnight to obviate plasma turbidity due to fats. The 12 ml. sample of blood drawn for each experiment was transferred to an oxalated tube and, after gentle mixing to dissolve the solid oxalate, was centrifuged to obtain the desired plasma. About 8 mg. of sodium oxalate per ml. of blood were required to prevent clotting in some of the pathological samples, this tendency to clot being due, apparently, to high fibrinogen contents. The plasma was diluted with phosphate-saline buffer to one-third its original concentration and dialyzed in Visking tubing against 2 liters of the buffer for 48 hours at refrigerator temperature. The buffer contained 5.76 ml. of 0.2M NaH_2PO_4, 94.24 ml. of 0.2M Na_2HPO_4 and 50 ml. of 3M NaCl in 1 liter of buffer solution, and was of pH 7.7 and of ionic strength 0.2.

Moving boundary electrophoresis experiments were made by means of a standard Tiselius-Klett apparatus with a single section, 11 ml. cell and at a potential gradient of 5 volt/cm. The relative concentrations of the plasma components were determined from the measured areas under the various peaks of the diagrams which were obtained by the enlargement of photographs, taken by the Longsworth scanning method (5), after electrophoresis for 5 hours. The diagrams were divided into sections representing the various plasma components by raising perpendicular lines from the baseline to the lowest point between adjacent peaks (6, 7). Tracings of ascending boundaries were used for calculation since a division between the "γ" and "δ" peaks was more readily made on the ascending than on the descending patterns. The areas under the "δ" peaks were disregarded in the calculations of the percentages of the various plasma constituents.

RESULTS

Since experiments were made on 36 rabbits, many of which were used in confirmatory studies, representative data only have been presented wherever possible. While in some instances electrophoresis runs were made on plasma from rabbits for which the white cell study was unavailable or incomplete, the reproducibility of the effect of neutron irradiation on white cell count and on plasma composition justified the inclusion of such data.

Normal White Blood Cell Count. The count for any one rabbit was found to vary considerably from day to day and, further, different rabbits were found to have different average normal values. The magnitudes of these

variations are shown in Table I in which mean values of the counts and corresponding standard deviations from the means are presented for each of 15 different animals.

Variation in White Blood Cell Count with Neutron Irradiation

55 n/day doses. A typical change in white blood cell count after irradiation was that shown by rabbit No. 22, given 55 n/day for 2 days; the data are presented graphically in Fig. 1a. The day-to-day variability of the normal count may be seen in the first part of the graph. After irradiation, an immediate rapid drop in count occurred; the minimum value was

TABLE I

Normal White Blood Cell Counts of Rabbits

Rabbit Number	Period Studied	Mean White Blood Cell Count	Standard Deviation
	days		
22	19	7,050	1,170
18	21	7,750	1,710
16	21	7,910	2,240
23	19	8,150	1,700
19	21	8,200	1,650
28	15	8,370	2,200
25	12	8,740	1,020
30	18	8,820	2,320
29	19	8,890	1,930
27	14	10,050	1,210
31	18	10,340	1,660
15	21	10,940	1,820
24	12	11,200	1,800
17	21	11,410	3,670
26	14	11,740	2,500
Average		9,300	±1,900

reached in 3 days and was followed by a recovery period which lasted about 1 month. Finally, this extended recovery period was followed by a further slight increase in count which, however, proceeded at a diminished rate. Re-irradiation of recovered animals produced a repetition of the response, as shown for rabbit No. 13 in Fig. 1b. Other rabbits, given total irradiation of 34–220 n over periods of 1–4 days, also exhibited a precipitous drop in white blood cell count immediately after irradiation, and required approximately 1 month for the return of the count to normal. Some animals, however, survived the irradiation for only a short time. This failure to survive began at total irradiations of 165 n and above, given in 55 n/day

exposures. One rabbit receiving 165 n died 3 weeks after cessation of irradiation, while another receiving the same dosage appeared to recover

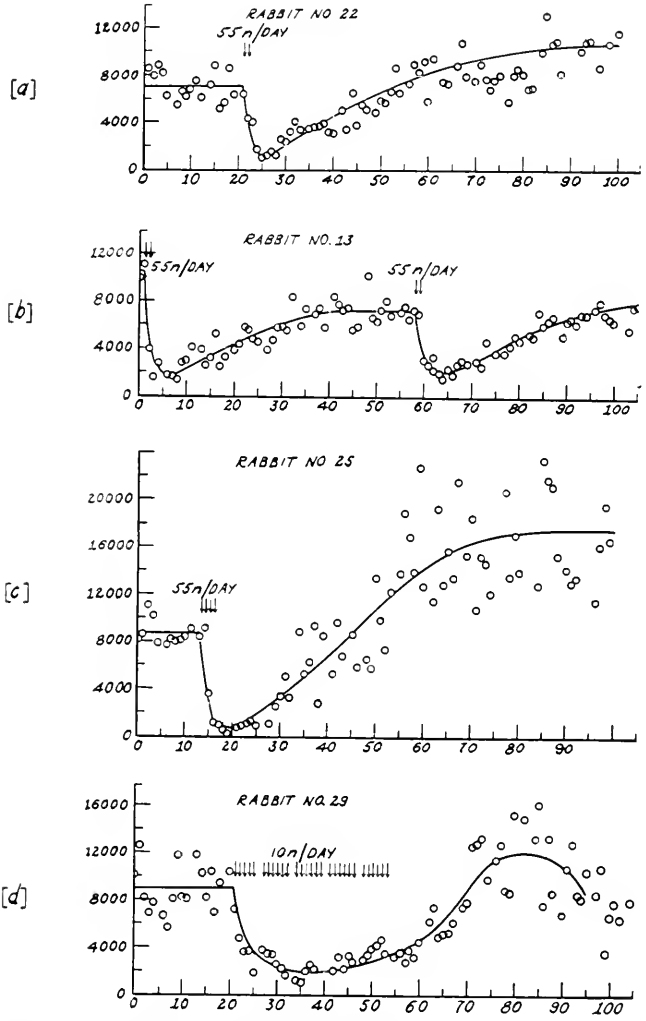


FIG. 1. Relationship between neutron irradiation of rabbits and their total white blood cell counts. The ordinate represents total white blood cell count; the abscissa, time in days. Arrows indicate days on which the irradiation was administered.

normal health. Of two rabbits given 220 n, one died 2 weeks after irradiation; the other, the white cell data for which are shown in Fig. 1c, made a temporary recovery for the first 5 weeks, after which, however, the white

cell count increased to an abnormally high value and the rabbit rapidly declined in health.

Repeated doses were found to result in much lower white blood cell counts than single doses, as shown in Table II.

10 n/day doses. The effect on the white blood cell count of irradiation with 10 n/day over an extended period is illustrated by the graph for rabbit No. 29 in Fig. 1d. As may be seen, this daily irradiation tended to maintain the count at low values. During recovery the white cell count was sometimes characterized by overcompensation followed by a final return to a normal level. Ten other rabbits, also given a total of 290 n in 10 n/day doses, showed essentially the same response, except for a varying survival period which ranged from 2 to 97 days after irradiation was completed. Some of the rabbits were irradiated with only one side exposed

TABLE II

Minimum White Blood Cell Values Resulting from Repeated Doses of Neutrons

Rabbit Number	Irradiation Treatment					Minimum White Blood Cell Values				
	1st day	2nd day	3rd day	4th day	Total	Individual				Average
	n	*n*	*n*	*n*	*n*					
20, 21	27	7			34		4,300	4,000		4,150
10, 11	55				55		2,400	2,900		2,650
16, 14	55	14			69		2,400	2,100		2,250
12, 13, 22, 23	55	55			110	900	1,600	1,100	1,200	1,200
26, 27	55	55	55		165		800	800		800
24, 25	55	55	55	55	220		250	400		325
29, 30, 31, 32, 33	10 n/day for 29 days				290	1,100	1,200	1,200	1,500	1,100	1,220

toward the source of irradiation while others were exposed on alternate sides on alternate days: this did not alter the effect on the white cell count. Preliminary studies with rabbits given 5 n/day gave qualitatively similar results, although complete experiments under these conditions were not made.

The effect of neutron irradiation on the white blood cell count of rabbits was found to resemble closely that on the white blood cell count of rats and mice (2, 3) resulting from irradiation with neutrons or with X-rays.

Variation in Body Weight with Irradiation. The general health of the rabbits, as indicated by body weight, revealed their varying sensitivities to neutron irradiation. Animals given large amounts of irradiation lost considerable weight and showed diminished appetite. Rabbits exposed to a dose of only 34 or 55 n exhibited rather uniform continuous growth as

illustrated by the growth curve in Fig. 2a for rabbit No. 10 given 55 n at two widely separated times. With a greater number of 55 n doses, some animals showed an immediate cessation of growth or loss of weight while others exhibited a continued growth, though only temporarily, in spite of irradiation. The latter effect is illustrated by the growth curve in Fig. 2b for rabbit No. 25.

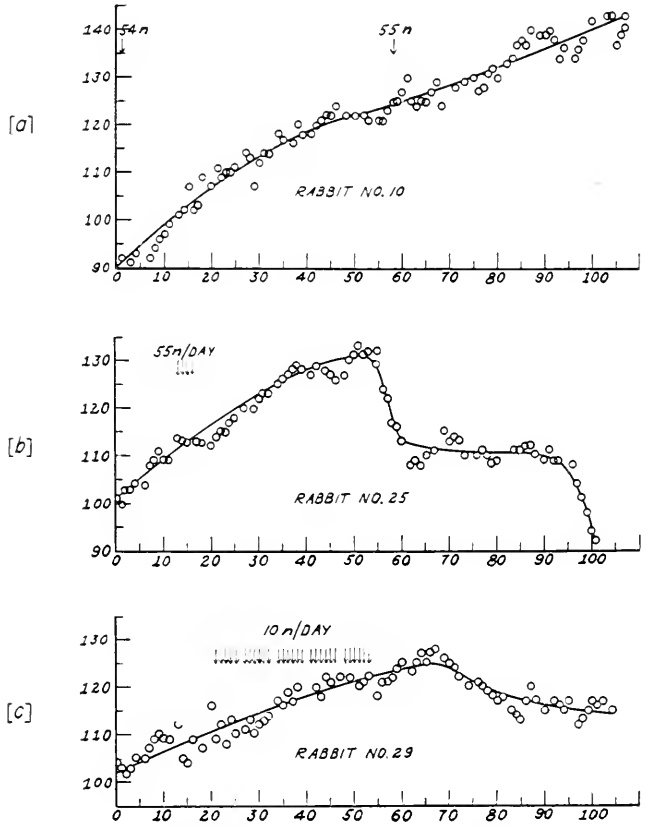


FIG. 2. Relationship between neutron irradiation of rabbits and their body weights. The ordinate represents weight in ounces; the abscissa, time in days. Arrows indicate days on which the irradiation was administered.

Irradiation with 10 n/day usually caused a decrease in growth rate followed by an actual decline in weight, as shown for rabbit No. 29 in Fig. 2c.

Physical Abnormalities Resulting from Irradiation. During the experiments, certain physical changes in the animals were so outstanding that they could not escape notice. One was alopecia, which first appeared about the nose and eyes, and finally spread over the entire bodies of the

animals. Rabbits given total irradiation small enough to permit complete recovery did not show appreciable alopecia: those given large amounts and surviving temporarily, however, exhibited alopecia and a generally scaly condition of the skin within a week or two after cessation of irradiation. Local loss of hair resulting from neutron irradiation over a limited body area has been demonstrated by Aebersold (8).

Animals given large amounts of irradiation lost considerable weight, showed diminished appetite and reduced activity and, when approaching death, failed to pass feces.

A consistent autopsy finding was diminished testicle size; this has also been observed with neutron-irradiated rats (9). Another common finding was the pronounced yellow color of the bone marrow, observed particularly in animals given 300 n at 10 n/day. Aplastic bone marrow has been found by Lawrence and Tennant (10) in neutron-irradiated mice and by Ely, Ross and Gay (9) in neutron-irradiated rats.

While the determination of the ultimate causes of death resulting from neutron irradiation was outside the scope of the present study it is significant that tissue destruction and infection have been proposed by others as probable factors (10).

The Electrophoretic Pattern of Normal Rabbit Plasma. Tracings of scanning diagrams obtained for representative normal plasmas are shown in Fig. 3a and Fig. 3b for rabbits Nos. 29 and 30, respectively. A component indicated by a peak between those of β-globulin and fibrinogen was of uncertain identity and was therefore labeled the ν-component; it may be a β-globulin.* The identity of the fibrinogen peak was established by virtue of its absence in corresponding electrophoresis diagrams of sera. The variation in the percentages of the different constituents present in the plasmas of 5 normal rabbits is shown in Table III.

Change in Plasma Electrophoretic Pattern due to Irradiation

55 n/day doses. Three rabbits, given 220 n over a period of 4 days, yielded plasmas 2–5 days after termination of irradiation, the electrophoretic patterns of which were superficially quite normal in appearance: on closer analysis, however, the patterns revealed a diminished average γ-globulin content. This is shown by a comparison of the data for rabbits Nos. 4, 3, and 5, in Table IV, and data for normal rabbits in Table III. The results, also shown in Table IV, for rabbits Nos. 8 and 9 given 275 n over a 5-day period, not only confirmed the above finding but suggested,

* This component has been reported to be one of the usual constituents of rabbit plasma, and has been referred to as "X-component" (11). Although not mentioned by others it is apparent in their published electrophoresis diagrams for rabbit serum and plasma (e.g., 12, 13).

in addition, that abnormalities in the plasma picture became magnified with increased total irradiation and with increasing time after cessation of irradiation. Thus, for rabbits Nos. 8 and 9, the percentage of albumin as

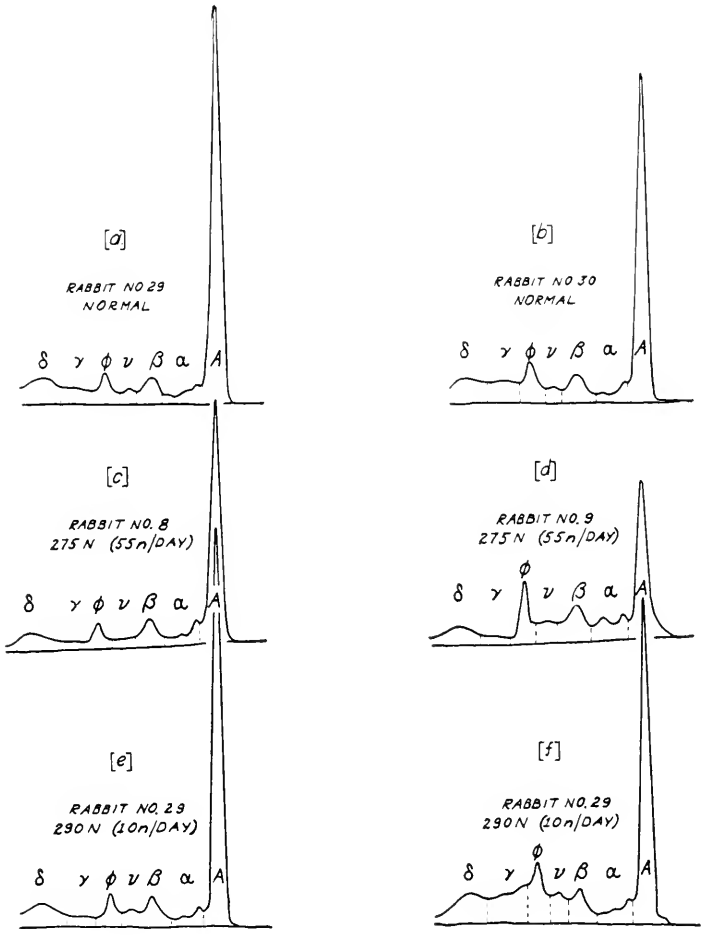


FIG. 3. Tracings of electrophoresis diagrams of blood plasmas of normal and irradiated rabbits. The symbol A represents albumin; α, α-globulin; β, β-globulin; ν, an unidentified component, possibly a β-globulin; ϕ, fibrinogen; γ, γ-globulin; δ, the "δ" boundary.

well as of γ-globulin diminished, while the percentages of the α- and β-globulins and of fibrinogen increased beyond the normal values.

The effect on the plasma of increased time between the completion of

irradiation and the withdrawal of blood for electrophoresis is illustrated by the scanning diagrams shown in Fig. 3c and 3d for the plasmas of rabbits Nos. 8 and 9, respectively. Both rabbits were irradiated together (receiving 275 n in five 55 n/day doses), but rabbit No. 8 was sacrificed one day, and rabbit No. 9 four days, after irradiation ceased: the blood for electrophoresis was obtained at these times. That the percentages of the plasma constituents varied with increasing time after irradiation may be seen by comparing the sizes of the peaks in the diagrams.

Rabbits Nos. 6 and 1 of Table IV, given 495 and 814 n, respectively, yielded plasmas which were still more abnormal but which also exhibited lowered γ-globulin and albumin, and increased α-globulin, β-globulin and fibrinogen contents.

10 n/day doses. The plasmas of rabbits administered neutron irradiation at the rate of 10 n/day gave relatively normal electrophoresis diagrams up to 2 weeks after cessation of irradiation, except for an unmistakable

TABLE III

Percentages of Electrophoretic Components in Plasmas of Normal Rabbits

Rabbit Number	Albumin	α-Globulin	β-Globulin	ν-Component	Fibrinogen	γ-Globulin
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
29	64.3	8.3	8.3	3.8	8.0	7.3
30	58.0	8.2	9.4	3.6	9.9	10.9
31	55.7	6.7	9.6	5.1	10.9	12.0
34	53.9	7.7	12.0	6.3	11.3	8.9
35	64.9	5.0	8.4	3.4	8.0	10.3
Mean	59.4	7.2	9.5	4.4	9.6	9.9

drop in the proportions of γ-globulin during the irradiation period. The lowest concentrations of γ-globulin observed were of the same order as those for animals given larger neutron doses per day. Subsequently, the γ-globulin percentage returned to, and then greatly exceeded the normal, while, concomitantly, the percentage of albumin diminished. These findings are demonstrated by a comparison of the data in Table IV for rabbits Nos. 29, 30, and 31 with those shown in Table III for the normal protein distribution in the plasma of the same rabbits. Typical of the electrophoresis diagrams corresponding to these slightly abnormal, and more definitely abnormal, protein distributions are those shown in Fig. 3 for plasma samples from rabbit No. 29: 3e shows the diagram obtained immediately after, and 3f that obtained 6 weeks after, the cessation of 290 n irradiation. The extent of the abnormalities may be seen by comparing these diagrams with that in Fig. 3a, which shows the electrophoresis diagram for the plasma of rabbit No. 29 before irradiation.

The gradual increase of γ-globulin to an unusually high concentration after the completion of irradiation was observed consistently with rabbits treated with 10 n/day for periods of about 30 days.

TABLE IV

Percentages of Electrophoretic Components in Plasmas of Irradiated Rabbits

Rabbit Number	Irradiation Treatment		Time After Irradiation	Albumin	α-Globulin	β-Globulin	γ-Component	Fibrinogen	γ-Globulin
	Per Day	Total							
	n	*n*	*days*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
4	55	220	2	62.9	6.4	9.9	4.2	8.6	8.0
3	"	"	4	62.5	6.1	11.3	3.7	10.8	5.5
5	"	"	5	59.3	9.9	9.9	5.4	9.3	6.3
8	"	275	1	60.3	8.2	12.1	7.1	8.5	3.9
9	"	"	4	41.5	14.6	16.9	9.0	14.6	3.3
6	"	495	2	42.3	9.7	19.5	9.7	13.5	5.2
1	*	814	1	42.4	10.5	20.6	7.5	16.1	3.0
29	10	100	0	62.1	8.1	11.5	4.6	7.1	6.6
	"	190	0	62.7	8.0	11.7	4.2	9.0	4.5
	"	290	0	65.3	7.8	9.9	4.8	8.6	3.5
	"	"	7	64.4	7.3	10.3	4.6	7.9	5.4
	"	"	14	61.2	7.3	10.5	4.8	9.6	6.6
	"	"	28	48.1	9.0	9.2	8.6	11.6	13.4
	"	"	42	48.6	7.4	10.1	6.4	14.0	13.4
30	10	100	0	66.1	4.4	10.6	3.9	8.0	7.0
	"	190	0	65.6	6.4	9.8	4.0	9.2	4.9
	"	290	0	64.0	5.7	10.6	4.4	10.1	5.2
	"	"	7	54.3	7.1	13.1	6.5	11.6	7.4
	"	"	14	58.2	6.1	10.4	5.1	11.4	8.9
31	10	290	21†	52.7	5.3	9.5	5.1	15.4	12.1
	"	"	91	47.9	6.3	8.5	7.1	11.4	18.8
	"	"	140	22.3	14.6	11.6	8.9	24.6	18.1

* Irregular treatment over a 5-day period, 400 n administered on next to the last day of irradiation.

† Electrophoretic analyses were not made before 3 weeks after irradiation ceased so that the animal would be available for a more protracted study.

Further, on many of the corresponding electrophoresis diagrams there were two small peaks between the fibrinogen and δ-boundary peaks, indicating the presence of two presumably γ-globulins in these plasmas. Peaks corresponding to two γ-globulins were not observed on the electro-

phoresis diagrams for normal rabbit plasmas, and the significance of the additional component in these plasmas obtained after irradiation is not known.

Extremely abnormal plasma protein distributions, namely low albumin concentrations but high concentrations of all the globulins and of fibrinogen, were observed for the animals in this group which were near death as a result of irradiation. This finding is illustrated by the data given in Table IV for rabbit No. 31, 140 days after completion of irradiation when the animal was close to death.

A comparison of these results for rabbit No. 31 with those for rabbit No. 1 (which died 1 day after irradiation ceased) brings out the importance of the rate of irradiation. It is apparent that sufficient time had not elapsed for the irradiation to produce maximum abnormality in the plasma before rabbit No. 1 died as a result of the irradiation.

Examined comprehensively, the results obtained in this study indicate that the size of the unit dose, as well as the total irradiation received, plays an important part in the response of animals to neutron irradiation.

Direct Irradiation of Plasma. A large sample of plasma from a single rabbit was diluted to one-third its original concentration with buffer, and aliquots in test tubes were subjected to irradiation doses of 0, 59, 440, 671, and 2144 n. After irradiation, the samples were each dialyzed against buffer and then examined electrophoretically. No significant differences were observed in the electrophoretic patterns of these samples, showing that direct neutron irradiation did not alter the protein distribution in the plasma.

DISCUSSION

The primary effect on the plasma of both the irradiation procedures used was a decrease in the γ-globulin concentration. The changes in γ-globulin concentration roughly paralleled the changes in total white cell count of the blood. This relationship for animals treated with large neutron doses per day is suggested by a comparison of the white blood cell data in Fig. 1 for rabbits given 55 n/day doses with the data in Table III for the γ-globulin concentrations of normal rabbits, and with those in Table IV for the γ-globulin concentrations of rabbits given 55 n/day. The correlation between the white cell count and the γ-globulin content of the plasma is strengthened by the results obtained with rabbits given 10 n/day doses, since in these experiments white cell counts and electrophoresis studies were made at several different intervals after irradiation and, furthermore, on the same animals. (See the representative white cell data for rabbit No. 29 in Fig. 1 and the data in Tables III and IV for the γ-globulin concentration in the plasmas of rabbits Nos. 29 and 30.)

In view of evidence presented by a number of workers that the γ-globulin of plasma originates in lymphocytes (14–17), a diminished white cell production might be expected to result in a lowered γ-globulin concentration in the plasma. Since the lymphocytes comprise as much as 30 to 50 per cent of the total white cells of rabbit blood (18), the actual drop in total white cell count, as indicated in Table II, is sufficiently great to require a marked diminution also in the lymphocyte fraction of the total count.

Had neutron irradiation caused a drop in white cell count by the breaking down of lymphocytes, an increase in plasma γ-globulin might reasonably have been expected (19). The fact that a decrease in γ-globulin resulted from irradiation suggests that the source of the globulin, namely, the lymphocytes themselves, was first affected, presumably by a diminished activity of lymphoid tissue, and that this brought about the observed diminution in γ-globulin content.

Additional and more profound departures from normal protein distribution were observed after sickness had overtaken those rabbits given neutron irradiation insufficient to cause death shortly after the irradiation ceased but sufficient to bring about eventual death. Thus, depletion in albumin together with increase in the proportion of the globulins and of fibrinogen was observed to be concomitant with decided loss in health of the animals. These later changes may have been associated with infection or with destruction of tissues of the liver, kidney, or other organs, as suggested by their similarity to changes which have been demonstrated by electrophoresis to occur in the plasma of humans with various diseases (20–27). The changes also resembled those obtained by electrophoresis experiments for protein-depleted dogs (28), suggesting that the malnutrition which resulted from loss of appetite after irradiation may have been a complicating factor. Consequently, an unequivocal interpretation of the effects of neutron irradiation as shown by the electrophoresis diagrams is not possible without further investigation.

SUMMARY

Electrophoresis studies have been made on plasma during and after neutron irradiation of rabbits. Comparisons were made of the effect of different irradiation procedures on plasma protein distribution.

Collateral studies were made of the changes in white blood cell count brought about by irradiation in order to relate the health of the animal after irradiation to an easily detected biological response.

The initial effect of the irradiation procedures used was a decrease in the γ-globulin concentration of the blood plasma, which paralleled roughly a simultaneous decrease in white cell count. For animals given large amounts of neutron irradiation in small doses the return to normal of the

γ-globulin concentration paralleled that of the white cell count. The observed changes in γ-globulin concentration may have occurred as a secondary effect of variation in lymphocyte number.

Subsequently, and concomitant with the appearance of marked losses in health of the animals, a depletion in albumin together with an increase in the globulins and fibrinogen occurred in the plasma. These plasma changes might have resulted in part from infection, starvation, or from the destruction of the tissues of liver, kidney, or other organs.

Direct irradiation of rabbit blood plasma produced no significant changes in the electrophoretic pattern of the plasma.

REFERENCES

(1) Tiselius, A., *Trans. Faraday Soc.*, **33**, 524 (1937).
(2) Lawrence, J. H., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 124 (1936).
(3) Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 543 (1936).
(4) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(5) Longsworth, L. G., *J. Am. Chem. Soc.*, **61**, 529 (1939).
(6) Tiselius, A., and Kabat, E. A., *J. Exptl. Med.*, **69**, 119 (1939).
(7) Longsworth, L. G., *Chem. Rev.*, **30**, 323 (1942).
(8) Aebersold, P. C., *Phys. Rev.*, **56**, 714 (1939).
(9) Ely, J. O., Ross, M. H., and Gay, D. M., Chapter 20.
(10) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
(11) McDonald, E. *J. Franklin Inst.*, **239**, 87 (1945), Figs. 4 and 5.
(12) Deutsch, H. F., and Goodloe, M. B., *J. Biol. Chem.*, **161**, 1 (1945).
(13) Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Immunol.*, **44**, 115 (1942).
(14) Kass, E. H., *Science*, **101**, 337 (1945).
(15) White, A., and Dougherty, T. F., *Endocrinology*, **36**, 207 (1945).
(16) Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E., *J. Exptl. Med.*, **81**, 73 (1945).
(17) Dougherty, T. F., Chase, J. H., and White, A., *Proc. Soc. Exptl. Biol. Med.*, **57**, 295 (1944).
(18) Scarborough, R. A., *Yale J. Biol. Med.*, **3**, 63 (1930).
(19) White, A., and Dougherty, T. F., *Ann. N. Y. Acad. Sci.*, **46**, 859 (1946).
(20) Longsworth, L. G., Shedlovsky, T., and MacInnes, D. A., *J. Exptl. Med.*, **70**, 399 (1939).
(21) Longsworth, L. G., and MacInnes, D. A., *J. Exptl. Med.*, **71**, 77 (1940).
(22) Gray, S. J., and Barron, E. S. G., *J. Clin. Investigation*, **22**, 191 (1943).
(23) Lewis, L. A., Schneider, R. W., and McCullagh, E. P., *J. Clin. Endocrinol.*, **4**, 535 (1944).
(24) Lewis, L. A., and McCullagh, E. P., *Am. J. Med. Sci.*, **208**, 727 (1944).
(25) McCullagh, E. P., and Lewis, L. A., *Am. J. Med. Sci.*, **210**, 81 (1945).
(26) Dole, V. P., and Emerson, K., Jr., *J. Clin. Investigation*, **24**, 644 (1945).
(27) Dole, V. P., Watson, R. F., and Rothbard, S., *J. Clin. Investigation*, **24**, 648 (1945).
(28) Zeldis, L. J., Alling, E. L., McCoord, A. B., and Kulka, J. P., *J. Exptl. Med* **82**, 157 (1945).

Chapter 15

ELECTROPHORESIS OF THE PLASMA OF DOGS IRRADIATED WITH NEUTRONS

By EDWARD B. SANIGAR

Near the conclusion of a study of the effects of neutron irradiation on the electrophoresis pattern of rabbit plasma (1) an opportunity occurred for the examination of the plasma of dogs which had been irradiated with small neutron doses daily over a prolonged period. In addition, four dogs were available for the study by electrophoresis of plasma changes resulting from much larger neutron doses over a very much shorter period of time.

While these opportunities did not permit a comprehensive study of the effect of neutron irradiation on the electrophoresis pattern of dog plasma, they yielded results which are both instructive and informative.

MATERIALS AND METHODS

Animals. The dogs whose plasmas were investigated in this study were males and females from mixed breeds of the Beagle type. Prior to, and during irradiation they were maintained on a diet of commercial dog food ("Friskies") and water, and all were in good health when irradiation was begun.

The animals examined were divided into three groups: Group A, normal, non-irradiated dogs used as controls for the Group B animals; Group B, those dogs which had received relatively small daily irradiation doses 6 days a week over a 12-month period, and Group C, those which were given high dosages over a short 4-day period. The dogs in Groups A and B were about 15 months old (except for dog B-7 which was 5 years and 3 months old) when the irradiation of the Group B dogs was commenced, and their weights were between 7.4 and 12.3 kg. with an average weight of 9 kg. Group C dogs were 27 months old with an average weight of 12 kg. at the time of their irradiation.

Irradiation. The production of the neutrons used, their energies, and the procedures and conditions of animal irradiation are described by Enns *et al.* (2).

The animals in Group B received their prescribed daily irradiation dose in one treatment lasting approximately 5 minutes, the difference in dosage rate being due to the position of the cage in which each animal was regularly placed for irradiation (2). The four dogs in Group C received 115-n

irradiation doses in single 6–7-hour exposures on each of 3 successive days, followed by a 55-n dose in 3 hours on the fourth day, cage No. 4 (2) being used for these irradiations. The control animals were placed in any available cages for 5 minutes 6 days a week for a year but received no irradiation. The daily irradiation dose, and total irradiation received by each dog are shown as part of Table I.

Preparation of Plasma Samples. To ensure clear, transparent plasma, blood samples were taken from animals from which all food, except water, had been withheld overnight. About 20 ml. of blood were obtained by venipuncture from the jugular or the radial vein, and immediately transferred to tubes containing dry lithium oxalate powder. The plasma obtained was stored in sterile vials close to the freezing coils in a refrigerator until used.

In preparation for electrophoresis, 5 ml. of plasma were added to 10 ml. of a phosphate-saline buffer solution of pH 7.7, of ionic strength 0.2, and of composition 5.76 ml. of 0.2M NaH_2PO_4, 94.24 ml. of 0.2M Na_2HPO_4 and 50 ml. of 3M NaCl in 1 liter of buffer solution. The diluted plasma was then placed in Visking tubing and the tube securely closed by tying in such manner that a slight pressure was put upon the plasma solution, thus assuring against the entry of any considerable quantity of water, with corresponding dilution of the plasma solution, during dialysis. The diluted plasma was dialyzed at refrigerator temperature for 40–50 hours against approximately 1600 ml. of the buffer solution, with occasional agitation.

Electrophoresis Measurements. Electrophoresis experiments were made with a standard Tiselius-Klett electrophoresis apparatus using a standard 11 ml. single section cell. During electrophoresis the current was maintained at 35 ma., resulting in a field strength of 5 volts/cm. in the cell.

The scanning photograph obtained in the usual manner (3) of the ascending (positive) arm of the cell after 6 hours of electrophoresis was taken for the determination of the relative amounts of the plasma constituents present, the positive arm usually giving clearer separation of the constituents and being free of anomalous effects due to slight precipitate formation which often occurred in the negative arm.

The enlarged tracing of the curve was divided into the various components by drawing an ordinate between the base line and the lowest point between adjacent peaks, following the procedure of Tiselius and Kabat (4) and Longsworth (5). The areas under the various peaks were measured with a planimeter, and the relative composition of the plasma evaluated by determining the percentage of the total area (excluding that of the delta boundary) contributed by each component.

TABLE I

Percentage Composition of the Plasmas of Normal and Neutron-irradiated Dogs

Dog	Sex	Irradiation: Cage Used	Irradiation: Daily	Irradiation: Total	Blood Drawn after Completion of Irradiation	Per Cent. of Electrophoretic Components: Alb.	α_1	α_2	α_3	α_4	Total α	β_1	β_2	Total β	ϕ	γ	Albumin Globulin Ratio
			n	*n*	*days*												
A 1	F	Any	0	0	–	55.1	6.4	4.1	4.3	2.9	17.7	7.3	4.6	11.9	10.7	4.6	1.6
2	M	"	0	0	–	53.2	7.7	4.8	4.5	4.0	21.0	4.3	4.5	8.8	10.4	6.7	1.5
3	F	"	0	0	–	51.9	8.0	2.4	4.6	3.3	18.3	6.4	5.9	12.0	11.6	6.3	1.4
B 1	F	1	0.012	3.7	1	49.3	7.2	5.1	3.7	1.6	17.6	2.7	7.7	10.4	16.5	6.1	1.4
2	M	1	0.012	3.7	1	56.6	5.8	3.4	2.5	3.4	15.1	2.2	4.9	7.1	13.9	7.4	1.9
3	M	2	0.06	18.7	1	48.5	5.3	3.8	4.8	3.6	17.5	3.0	7.4	10.4	17.3	6.4	1.4
4	F	2	0.06	18.7	1	47.3	7.3	3.6	4.2	2.5	17.6	3.9	5.3	9.2	19.3	6.4	1.4
5	M	3	0.11	33.0	1	54.1	4.2	3.1	6.0	3.9	17.5	7.3	6.5	13.8	7.6	7.0	1.4
6	M	3	0.11	34.3	1	49.7	5.0	1.2	3.9	3.4	16.5	2.6	5.2	7.8	18.1	7.9	1.5
7	F	4	1.7	507	1	46.3	7.7*		6.6		14.3	21.0		24.0	11.0	4.4	1.1
C 1	M	4	3 × 115 1 × 55	400	† 3 6	51.3 49.3 24.8	8.0 6.5 1.1	3.8 3.2 4.7	5.3 6.8 21.2	4.7 4.6 10.2	21.8 21.1 37.2	4.4 3.0 6.2	4.1 7.6 10.6	8.5 10.6 16.8	9.4 11.1 17.7	5.9 7.6 3.5	1.5 1.3 0.1
2	M	4	3 × 115 1 × 55	400	† 3 6	46.7 43.1 (Died)	7.5 5.4	3.0 3.7	3.9 6.6	2.1 1.6	16.5 20.3	9.6 7.1	10.2 10.0	19.8 17.1	9.0 11.6	8.1 4.9	1.1 1.0
3	F	4	3 × 115 1 × 55	400	† 3 6	43.9 44.5 27.6	9.9 9.1 1.1	5.9 3.4 6.0	7.7 6.3 24.2	3.0 1.1 6.2	26.5 23.2 37.5	4.9 4.7 4.1	5.6 8.1 9.9	10.5 12.8 14.0	12.7 12.8 15.0	6.3 6.8 5.8	1.0 1.0 0.5
4	F	4	3 × 115 1 × 55	400	† 3 6	43.9 45.3 32.1	8.5 9.1 4.6	3.9 3.9 7.6	5.7 6.7 15.2	3.9 3.6 3.9	22.0 23.3 31.3	4.7 4.2 2.0	7.7 6.9 12.0	12.4 11.1 14.0	14.2 11.7 16.3	7.5 5.6 6.0	1.0 1.1 0.6

* 3-hour curve: boundary separation incomplete.

† Immediately before irradiation.

RESULTS

The control animals of Group A maintained or increased their weights over the 12-month period. The dogs in Group B, except for dog B-7, maintained their weights during irradiation and survived treatment satisfactorily. Group C dogs, receiving large neutron doses daily, lost weight rapidly following irradiation, and succumbed to the effect of the irradiation 6–8 days after the final exposure.

The curves obtained after 6 hours of electrophoresis showed separation of the plasmas into albumin, four α-globulins, two β-globulins, fibrinogen, and γ-globulin components. The percentage of each constituent, as well as the albumin/globulin ratio, is presented in Table I. Because of the

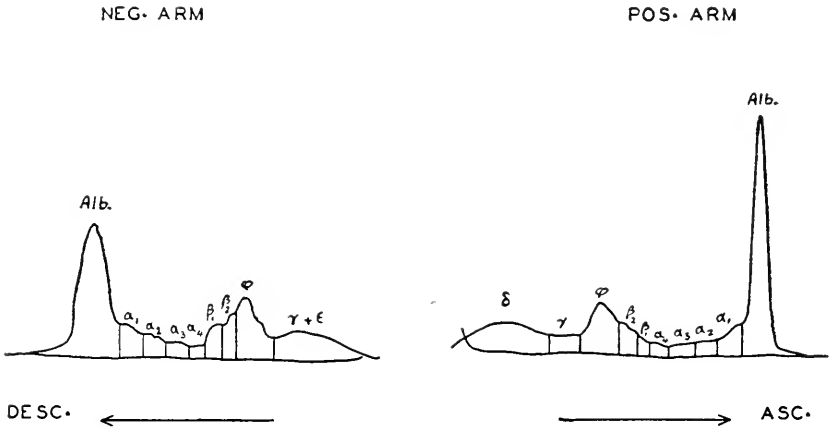


FIG. 1. Electrophoresis pattern of the plasma of dog B-4 after prolonged low-dosage neutron irradiation.

probability that an appreciable error is introduced into the determination of the values of individual constituents by the difficulty of dividing unequivocally, for measurement, the area due to a group of unsatisfactorily resolved peaks, the total percentages of α-globulin and of β-globulin are also included in Table I.

An examination of the figures given in Table I for Group A (normal) and Group B (low dosage) dogs indicates that the distribution of the components in the plasmas of the Group B dogs (excluding dog B-7) differs little from the normal except for an increased fibrinogen content. The table also shows the essential constancy of the albumin/globulin ratios for the plasmas of the two groups. Dog B-7, which received considerably larger total neutron irradiation and at a higher rate, showed a somewhat

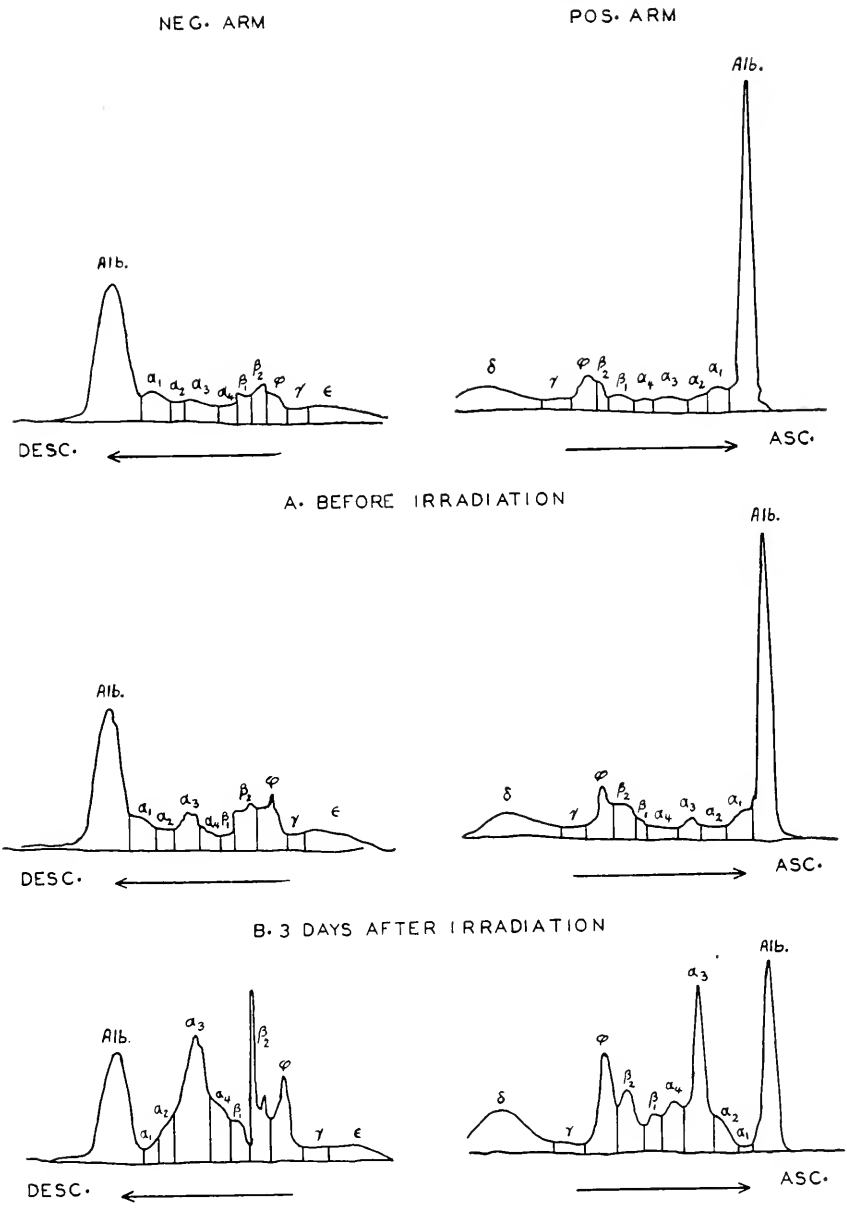


FIG. 2. Electrophoresis patterns of the plasma of dog C-1 before and after high-dosage neutron irradiation.

decreased α-globulin content, an increased β-globulin content and a slightly decreased γ-globulin content.

A typical electrophoresis diagram for the Group B dogs is that for dog B-4 given in Fig. 1. No diagrams are given for the Group A dogs since they all closely resemble that shown in Fig. 2 (A), the electrophoresis diagram for dog C-1 before irradiation.

Much more striking are the results obtained for the dogs given a very much higher rate of neutron irradiation (Group C dogs). It will be seen from Table I that with these dogs there are only small differences in composition between the pre-irradiation plasmas and those obtained 3 days after irradiation was stopped, and the value of the albumin/globulin ratio 3 days after irradiation is the same as before irradiation. The plasmas obtained 6 days after the cessation of irradiation, however, have lowered albumin contents and slightly lowered γ-globulin contents, with greatly increased α-globulin contents and increased β-globulin and fibrinogen contents, as compared with the corresponding plasma before irradiation. Closer examination reveals the increase in total α-globulin content to be due largely to the pronounced increase in the α_3-fraction, although the α_2- and α_4-fractions also show tendencies to higher than normal values: opposed to this is the decrease in the α_1-globulin values. Of the β-globulins, the β_1-fraction shows a definite tendency to decrease while the β_2-fraction increases, the result being an increase in the total β-globulin concentration of the 6-day plasma. Further, the decrease in albumin content and the corresponding increase in total globulin content (α, β, and γ) are shown in the much decreased value of the albumin/globulin ratio for these plasmas.

A typical series of electrophoresis diagrams for the dogs in Group C is given in Fig. 2 and serves to emphasize the changes in plasma composition described above.

DISCUSSION

The similarity of the electrophoresis diagrams and of the values of the albumin/globulin ratios for the Group B dogs to those of the Group A dogs is indicative of the very slight effect, if any, which relatively low neutron doses, even though continued for a prolonged period, have on the general health of the animals. The deviations from the normal values shown by dog B-7, which received considerably more irradiation than the other dogs in this group, indicate that a prolonged irradiation using a sufficiently large daily dose will result in disturbances in the blood protein balance.

The 400 n of irradiation given over a 4-day period (three daily doses of 115 n followed by one 55-n dose) caused a much greater plasma disturbance and had a much greater general effect on the animal than did a larger total

irradiation (507 n) given in 1.7 n daily doses over a year's time (*cf.* similar effects with rabbits (1)). This again emphasizes the importance of the rate at which neutron radiation is given.

The most apparent deviation from normal was the high value for the total α-globulin concentration shown by the plasmas obtained 6 days after the completion of the heavy neutron irradiation. The increase appears primarily in the α_3-globulin, although the α_2- and α_4-globulin constituents also show consistent increases whereas the α_1- globulin shows a decrease.

Much published work has shown that a very early effect of neutron irradiation is the pronounced reduction in the white cell count of the blood (6) indicating a disturbance of the blood producing system. Further, aplastic bone marrow has been reported (7) in animals subjected to neutron irradiation, and a high proportion of yellow bone marrow has been observed consistently in rabbits which have survived for some time after neutron irradiation (1). Also, the electrophoresis pattern of the bone marrow of neutron-irradiated rabbits has been shown to differ from that obtained for the bone marrow of non-irradiated rabbits (8). The yellow color of the bone marrow of dog C-2 has been given by Ross and Ely (9) among their pathological findings on the Group C dogs* after irradiation. Their clinical report on these Group C dogs reveals that the irradiation caused marked effects such as loss of appetite, loss of weight, subcutaneous hemorrhages and edema, in addition to leukopenia and destruction of lymphoid tissue. It is possible that such pronounced effects of neutron irradiation could result in the increased globulin percentages observed electrophoretically, although there appears to be no *a priori* reason why the α-globulin, and particularly the α_3-globulin, should be most affected. The tendency to a decrease in γ-globulin following irradiation has also been observed for neutron-irradiated rabbits (1) and could, possibly, be due to damage to the lymphatic system or to bone marrow as a result of irradiation.

An alternative explanation of the observed abnormalities of the plasmas obtained from the Group C dogs 6 days after irradiation ceased can be based on the fact that during the first day of irradiation the food intake of the animals dropped markedly, and after completion of irradiation the dogs did not eat any of the food provided (9). The final plasma samples obtained from dogs C-1, C-3 and C-4 were, therefore, those of dogs which had not taken any food for 6 days. Zeldis, Alling and their co-workers (10) have shown that the electrophoresis plasma patterns for protein-depleted dogs maintained for some 10 or more weeks on a low protein diet show a decrease in albumin while the electrophoretic globulin areas increase.

* These dogs in Group C are the four dogs studied by Ross and Ely (9). In the two studies dog C-1 and dog 1 are the same animal, etc.

They found such increases to result largely from elevated α-globulin peaks and, further, to be associated with the elevated plasma lipid levels which they observed in protein-depleted dogs.

On the basis of the present few experiments, and in the absence of knowledge of the effect of 6-day starvation on the electrophoretic composition of the plasma of a normal dog, it is not reasonable to attempt to decide between the two suggested explanations.

The increased plasma protein nitrogen with increasing time after irradiation observed for these dogs, is, it is suggested (9), due to tissue damage resulting from the effects of the irradiation; it does not seem plausible that it should be due to starvation. On the other hand, the observed increase in total α-globulin could be the result of starvation brought about as a secondary effect of the irradiation. It is also possible because of the extended involvement of so many of the body organs (9) that the profound disturbance of the bodily functions brought about by the irradiation, as well as the starvation produced as a secondary effect, may combine to give the observed abnormalities in the electrophoresis patterns.

SUMMARY

Electrophoresis patterns for the blood plasmas of neutron-irradiated dogs have been found to be essentially normal when the irradiation was of low intensity even though such irradiation was continued for a prolonged period. Abnormal plasma protein distribution occurred when somewhat higher dosage rates were continued over an extended period. High total neutron irradiation given at a high dosage rate resulted in a decreased albumin content coupled with a pronounced rise in total α-globulin content (the increase being almost entirely in the α_2-component), a decided rise in the total β-globulin content, and a decrease in γ-globulin content.

REFERENCES

(1) Sanigar, E. B., Miller, G. L., and Maddox, M. N., Chapter 14.
(2) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(3) Longsworth, L. G., *J. Am. Chem. Soc.*, **61**, 529 (1939).
(4) Tiselius, A., and Kabat, E. A., *J. Exptl. Med.*, **69**, 119 (1939).
(5) Longsworth, L. G., *Chem. Rev.*, **30**, 323 (1942).
(6) Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 543 (1936); Lawrence, J. H., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22**, 124 (1936).
(7) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
(8) Keeici, L. E., Leitch, J. L., and Sweeny, L., Chapter 12.
(9) Ross, M. H., and Ely, J. O., Chapter 19.
(10) Zeldis, L. J., and Alling, E. L., *J. Exptl. Med.*, **81**, 515 (1945); Zeldis, L. J., Alling, E. L., McCoord, A. B., and Kulka, J. P., *J. Exptl. Med.*, **82**, 157 and 411 (1945).

Chapter 16

THE ULTRAVIOLET ABSORPTION SPECTRA OF PLASMA AND HEMOGLOBIN SOLUTIONS FROM THE BLOOD OF NEUTRON-IRRADIATED RABBITS AND DOGS

By EDWARD B. SANIGAR

There is much published work showing that the irradiation of an animal by neutrons causes a profound involvement of body organs with considerable disruption of normal bodily functions (e.g., 1). Some of these disturbances are reflected by abnormalities in the blood and blood plasma of the animal. For example, the plasmas of animals subjected to neutron irradiation of sufficient intensity and magnitude show deviations from the normal in their electrophoretic patterns (2, 3, 4) even though neutron irradiation of normal rabbit plasma *in vitro* is without effect (3).

Although blood plasma contains a number of fractions whose characteristic properties enable them to be distinguished by chemical or by electrophoretic means, it shows only one peak on the ultraviolet absorption curve. The position of the maximum point of this peak, approximately 2800 Å, as well as the shape of the absorption curve, is the same for serum and plasma from different animal species (e.g., 5).

Since the ultraviolet absorption of plasma has been established as due to the amino acid constituents of plasma, particularly the aromatic amino acids tryptophane, tyrosine, and phenylalanine (6), the degradation of plasma proteins even to the corresponding amino acids would not be expected to alter appreciably the shape of the absorption curve or the position of its maximum. No such profound degradation of plasma proteins is, however, indicated by the electrophoresis curves for the plasmas from neutron-irradiated animals.

Further, irradiation of serum albumin in solution with 29,000 Roentgen units of soft X-rays (Cu target, 100,000 volts) has been shown to be without effect on its ultraviolet absorption spectrum (7). While the effects of neutron irradiation on the animal body are much more pronounced than those of X-rays, the two forms of radiation are similar, producing similar bodily disorders and radiation sickness (1a).

It is thus possible to establish by argument the improbability of the neutron irradiation of an animal causing any change in the ultraviolet curve of its plasma or serum. Nevertheless it was thought desirable to test the validity of this conclusion by the determination of the ultraviolet

absorption spectra of the blood plasmas from some of the numerous irradiated animals available.

The probable effect of the neutron irradiation of an animal on its hemoglobin and, therefore, on the absorption spectrum of the hemoglobin does not, however, appear to be as easily predictable. While red blood cells have been found to be radioresistant, sufficiently large doses of X-radiation cause increased permeability of the cells with increased tendency to hemolysis (8). There also is some evidence, though not completely substantiated, that therapeutic doses of X-rays bring about an increase in average red blood cell volume and, further, that irradiation produces a wave of red blood cell regeneration (8). There does not appear to be any similar published evidence as to the effect of neutron irradiation on red blood cells. Neither has any statement been found of the effect, if any, of X-radiation or neutron radiation of an animal on blood hemoglobin itself.

It does not seem probable that such irradiation would affect hemoglobin, so that no difference between the absorption spectra of hemoglobin obtained from neutron-irradiated and from non-irradiated animals would be expected. The availability of irradiated animals and the rapidity with which absorption data are obtainable by means of echelon cells, again made a test of the conclusion worthwhile.

EXPERIMENTAL

Animals. All the rabbits used to provide blood for this work were male white rabbits weighing not less than four pounds, maintained on a diet of "Purina Rabbit Pellets" and water. Except for the "normal" (i.e., untreated and non-irradiated) animals, the rabbits had been used in the course of another study having as its object the determination of the effects of mononucleotides on the white blood cell count of normal and irradiated rabbits. Consequently some of the rabbits whose bloods were used to obtain the absorption data reported here had received daily intramuscular injections of a mononucleotide mixture ("Pentnucleotide", Smith, Klein and French, Philadelphia, Pa.) or of yeast adenylic acid (Schwartz Laboratories, Inc., New York, N. Y., lot number HA 4525) in addition to neutron irradiation. Others had received the injections but no irradiation, while still others had received neutron irradiation without any injections.

Blood samples from two dogs used in another study (4) (dogs C-1 and C-4, 3 and 6 days after the completion of their irradiation) were also examined.

Irradiation. The details of neutron production and of irradiation have been described elsewhere by Enns *et al.* (9). The animals received their daily neutron irradiation in approximately 2 hours usually in one continuous dose although some received intermittent irradiation (over approximately the same time) the interruptions being necessitated by the removal of

animals irradiated for other studies. Cages 4 and 5 (9) were used as the containers for the rabbits during irradiation.

Absorption Spectra. Absorption spectra were obtained using Hilger echelon cells (10) with a medium-sized Bausch and Lomb quartz spectrograph by technique previously described (11). The light used was from a water-cooled, low-voltage hydrogen arc (12), giving a continuous spectrum from about 1850 Å to 5000 Å. Photographs were taken with Eastman 33 plates.

The actual distances on the photographic plates were: ultraviolet, 1900 Å to 4000 Å, 15.6 cm.; 3000–5000 Å, the range covered by the hemoglobin absorption, 7.25 cm.; and 3700–4700 Å, the spread of the main peak of hemoglobin, 2.5 cm. Consequently, the position of the hemoglobin absorption maximum could not be determined with the same accuracy as could the plasma protein maximum, but the convenience of using the same apparatus for both purposes outweighed this consideration for this exploratory work.

Plasma and Hemoglobin Solutions. The blood (about 5 ml.) was obtained by heart puncture and placed in a tube containing dry lithium oxalate powder as an anticoagulant. Food, except water, was withheld from the animals overnight before bleeding so that none of the plasma showed any turbidity. After centrifuging the blood, the bulk of the plasma was pipetted into sterile serum vials, after which the remainder of the plasma, the white cells and some of the red blood cells, were pipetted off and discarded. The remaining red blood cells were stirred up and used in the preparation of the corresponding hemoglobin solution.

The plasmas were diluted to one-sixth their original concentrations with distilled water and used without further preparation. The hemoglobin solutions were prepared by adding 0.5 ml. of red blood cells to distilled water to make 100 ml. of solution in a volumetric flask, thoroughly mixing and allowing the solutions to stand at room temperature for 1 hour, followed by the centrifuging of about 5 ml. of the solution to remove cell debris. The above concentrations were chosen so that complete, unbroken absorption curves could always be obtained.

Except during the time required for the preparation of the solutions, the blood and the solutions were kept at refrigerator temperature until used. The absorption spectra were generally obtained within 48 hours after the blood was drawn.

DISCUSSION

The results obtained, together with the pertinent experimental data, are given in Table I. Two absorption curves are shown in Fig. 1: "A" for the plasma solution, "B" for the hemoglobin solution obtained from the

TABLE I

Absorption Maxima

Rabbit No.	Injections			Irradiation				Blood Drawn after Irradiation	Wave length of Maximum Absorption	
	Compound	Daily Dose	Duration	Daily Except Sundays	Duration	Total	Cage No.		Plasma	Hemoglobin
			days	*n*	*days*	*n*		*days*	Å	Å
Untreated—Non-irradiated										
1	none	—	—	none	—	—	—	—	2770	4130
2	"	—	—	"	—	—	—	—	2780	4140
3	"	—	—	"	—	—	—	—	2780	4150
4	"	—	—	"	—	—	—	—	2770	4145
5	"	—	—	"	—	—	—	—	2760	4150
6	"	—	—	"	—	—	—	—	2770	4150
Untreated—Irradiated										
7	none	—	—	5	72	300	4	68	2785	4130
8	"	—	—	5	72	300	4	68	2780	4140
9	"	—	—	5	72	300	4	41	2795	4120
10	"	—	—	10	35	300	5	62	2790	4140
11	"	—	—	10	37	300	5	36	2790	4130
12	"	—	—	10	37	300	5	76	2780	4130
13	"	—	—	14	24	294	4	69	2795	4135
Treated—Non-irradiated										
14	Pentnucleotide	2 cc.	35	none	—	—	—	32[1]	2780	—
15	Adenylic Acid	100 mg.	43	"	—	—	—	44[1]	2780	4135
16	" "	200 mg.	34	"	—	—	—	44[1]	2785	4135

Treated—Irradiated											
17	Adenylic Acid		100 mg.	71	5	70	290	4	0	2800	4130
18	"	"	100 mg.	68	5	67	275	4	0	2800	4440
19	"	"	100 mg.	60	5	59	240	4	0	2795	4130
20	"	"	100 mg.	52	10	1st 35	300	5	17	2800	—
21	"	"	100 mg.	15	10	14	110	5	0	2800	4130
22	"	"	100 mg.	22	10	21	180	5	0	2795	4120
Irradiated[2]											
Dog C-4	none			—	3 × 115 1 × 55	1	400	4	3	2795	4145
	"			—	"				6	2795	4130
Dog C-4	"			—	"	4	400	4	3	2820	4135
	"		—	—	"				6	2790	4140

[1] Days after treatment.

[2] For the electrophoresis patterns of the plasmas of these dogs see Chapter 15, and for the pathological findings at autopsy see Chapter 19.

blood of dog C-1 (4) 6 days after irradiation ceased. These two absorption curves are presented, first, because they are typical of all the curves, including those for the rabbits, obtained in this study, secondly, because the abnormal plasma protein distribution of this same plasma (as determined by electrophoresis) is shown in Fig. 2e of a previous chapter (4), and thirdly, because even though the dogs received much more intense neutron irradiation than did any of the rabbits, resulting in marked pathological abnormalities (1e), the curves are normal in shape and position of the maxima.

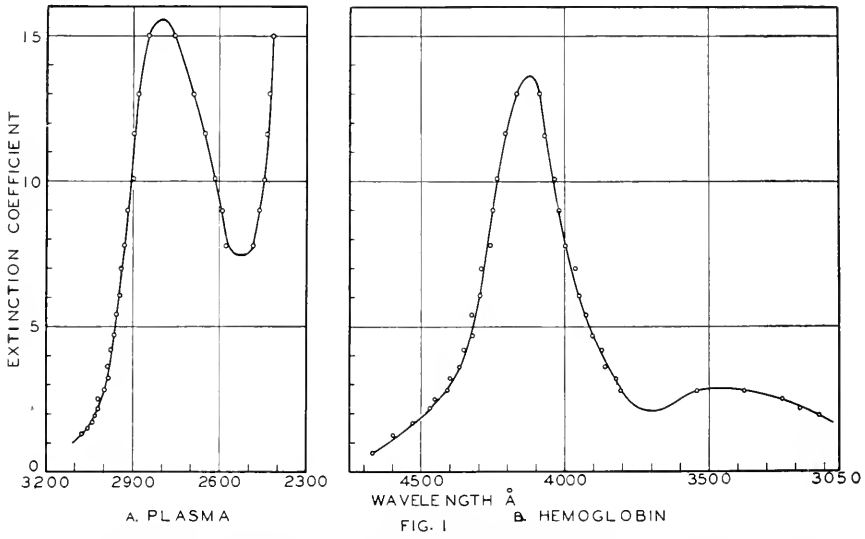


FIG. 1

FIG. 1. Absorption spectra of plasma and hemoglobin from the blood of a neutron-irradiated dog.

The absorption curves of all the plasma solutions examined showed single maxima at about 2800 Å with minima at about 2540 Å. Similarly, the curves for all the laked red blood cells (hemoglobin solutions) showed pronounced maxima at about 4140 Å and small maxima at about 3450 Å. The curves obtained for the blood hemoglobin solutions closely resembled, although they did not exactly duplicate, the curve for Pfanstiehl hemoglobin published by Reinhard (13).

Wave lengths of the absorption maxima for the plasma and hemoglobin solutions examined are given in Table I. The variation in the wave length of maximum absorption for the plasmas, and the very slight shift to higher than normal values for the plasmas of treated animals, or of irradiated animals (which can be observed on very close examination of Table I) are

ascribed to observational errors inherent in the method and to possible ambiguity of the exact position of the maximum, as obtained by drawing the best smooth curve through the experimentally determined points, rather than to any established trend resulting from irradiation or treatment.

The results obtained are, therefore, presented in substantiation of the conclusions that neutron irradiation of animals is without definite effect on the light absorption of the plasma or of the hemoglobin from the blood. They also show, secondarily, that intramuscular injections of yeast adenylic acid or of a mononucleotide mixture are without effect on the absorption curves of plasma or of hemoglobin from blood.

SUMMARY

Light absorption determinations have led to the conclusion that neutron irradiation of animals, with or without the injections of substances capable of influencing the white blood cell count, is without effect on the absorption spectrum of the plasma or of the hemoglobin of the blood.

REFERENCES

(1) a. Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
b. Snell, G. D., and Aebersold, P. C., *Proc. Natl. Acad. Sci.*, **23**, 374 (1937).
c. Lawrence, E. O., *Radiology*, **29**, 313 (1937).
d. Aebersold, P. C., and Lawrence, J. H., "Annual Review of Physiology", Stanford University, **4**, 25 (1942).
e. Ross, M. H., and Ely, J. O., Chapter 19.
(2) Krejci, L. E., and Sweeny, L., Chapter 13.
(3) Sanigar, E. B., Miller, G. L., and Maddox, M. N., Chapter 14.
(4) Sanigar, E. B., Chapter 15.
(5) Lewis, J. S., *Proc. Roy. Soc. London*, **93B**, 178 (1921–22).
(6) a. Stenström, W., and Reinhard, M., *J. Biol. Chem.*, **66**, 819 (1925).
b. Coulter, C. B., Stone, F. M., and Kabat, E. A., *J. Genl. Physiol.*, **19**, 739 (1939).
(7) Sanigar, E. B., Krejci, L. E., and Kraemer, E. O., *Biochem. J.*, **33**, 1 (1939).
(8) Warren, S., and Dunlap, C. E., *Arch. Path.*, **34**, 562 (1942).
(9) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(10) Twyman, F., Spencer, L. J., and Harvey, A., *Trans. Optical Soc. London*, **33**, 37 (1931–32); Twyman, F., *Proc. Phys. Soc. London*, **45**, 1 (1933).
(11) McDonald, E., *J. Franklin Inst.*, **221**, 103 (1936).
(12) Allen, A. J., and Franklin, R. G., *J. Optical Soc. Am.*, **29**, 453 (1939).
(13) Reinhard, M. C., *J. Genl. Physiol.*, **11**, 1 (1927).

Chapter 17

SOME PHYSIOLOGICAL RESPONSES OF RATS TO NEUTRON IRRADIATION

By J. O. ELY and M. H. ROSS

In the course of investigations concerning the effects of neutron irradiation on rats, a loss of weight following irradiation was observed. Food consumption and animal activity also appeared to be reduced. Because gastric symptoms are frequently associated with X-irradiation in human beings, it was considered possible that neutron irradiation might have similar effects on animals and that the loss in weight would be explained on the basis of reduced food consumption and the reduced food consumption might be attributed to some disturbing effect of neutron irradiation on gastrointestinal function.

EFFECT OF NEUTRON IRRADIATION ON FOOD INTAKE AND WEIGHT

In order to determine the effect of neutron radiation on the food consumption and weight of rats, 3 groups were studied. The animals of each group averaged 128 grams in weight, and were maintained on a diet of Purina Fox Chow with meat meal. Group I, 10 rats, was given a single dose of 56.4 n in 1 hour in Box No. 7 (Enns *et al.* (1)). Group II, 10 rats, was not irradiated, but was placed in the irradiation box for the length of time required to irradiate the animals of Group I. These 2 groups were allowed free access to food and water, and their average daily food intake, determined each day at the same hour, was recorded for a period of 2 weeks before irradiation and for 16 days after irradiation. Group III, 5 rats, was not irradiated but its food consumption was restricted each day to the amount consumed by the animals of Group I during their post-irradiation period.

The average daily food consumption of the non-irradiated and irradiated groups was similar during the pre-irradiation period. Beginning immediately after irradiation there was a sharp drop in the food consumption of the irradiated animals, Group I (Fig. 1A). The maximum reduction in food intake, about 44 per cent of pre-irradiation consumption, was reached 2 days after irradiation. Thereafter the daily food intake increased gradually until, at the 8th post-irradiation day, the pre-irradiation level was reached.

After the food consumption had reached the pre-irradiation level, it remained more or less uniform. The food consumption of non-irradiated

Group II, whose food intake was not restricted, continued to rise throughout the whole period.

During the pre-irradiation period the average weight increase of the rats of the 3 groups was identical. Immediately after irradiation there was a loss in weight in the irradiated animals (Fig. 1B). The maximum loss in weight, 6 per cent, was reached on the third day after irradiation. Thereafter the average weight of the irradiated animals increased daily and paralleled that of the non-irradiated animals. The average weight of the irradiated animals, however, remained approximately 6 per cent below that of the non-irradiated ones. The average weight of the rats of Group III, which were not irradiated but were limited in food consumption to the

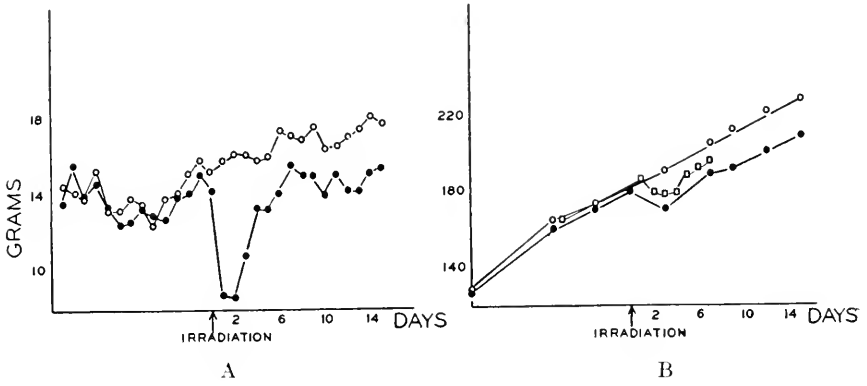


FIG. 1A AND B. The effect of 56.4 n on A) daily food consumption and on B) weight of rats. ●Irradiated. ○ Control. □Non-irradiated animals: food intake restricted to amount consumed by irradiated animals.

amounts eaten by the animals of Group I, showed a corresponding loss. The greatest weight reduction of the animals of Group III was reached on the 3rd day and differed by only 0.7 per cent from that of Group I rats.

The reduction in the growth rate following neutron irradiation was temporary, and the rate of growth soon paralleled that of the control rats. The actual weight, however, remained below that of the controls in amount equal to the weight loss immediately following irradiation. In this sense the animals failed to recover completely. The reduced food consumption appeared to be responsible for the loss in weight following 56.4 n irradiation.

EFFECT OF NEUTRON IRRADIATION ON GASTRIC DIGESTION

The reduction in food consumption noted in rats following neutron irradiation indicated a disturbance in gastrointestinal function. The effects of neutrons on the digestive process in the stomach of the rat, there-

fore, were investigated with special reference to the total fluid volume and the total solids in the stomach, and to the pH, the total acidity and the peptic activity of the gastric juice.

Both male and female rats, averaging approximately 200 grams in weight, were used. In each of 5 experiments one half of the rats was irradiated (1) in 1 hour with 56.4 n in Box No. 7, the other half serving as controls. The rats were fasted for 24 hours prior to irradiation and for 24 hours following irradiation and then given a test meal of finely powdered dried toast in distilled water, 0.8 g. of toast in a total volume of 4 cc., except where otherwise stated. At intervals after administering the test

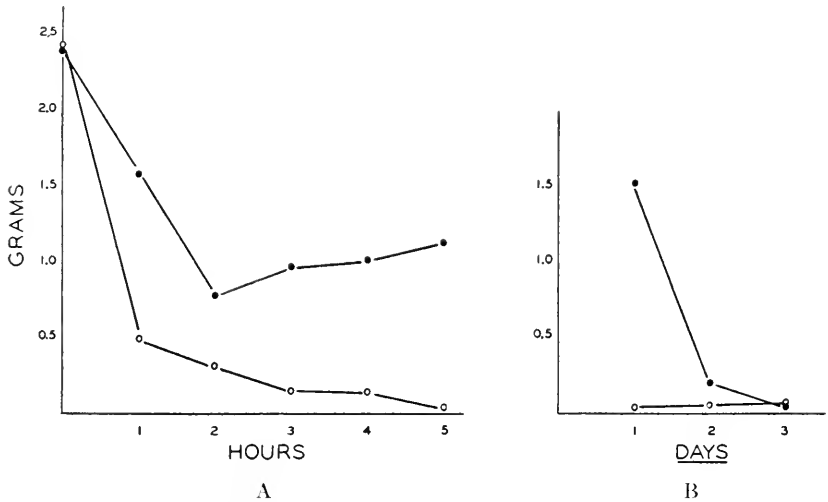


FIG. 2A AND B. The effect of 56.4 n on the amount of solid residue in the stomachs of rats A) following administration of test meal and B) fasted 24 hours before determinations. ● Irradiated. ○ Control.

meal by stomach tube, groups of 3 to 5 of the irradiated and of the control rats were decapitated, their stomachs removed and the contents examined.

Residual Solids. The average amount of solid residue (dry) remaining in the stomachs of each of the 5 groups of irradiated rats tested at hourly intervals following the administration of the test meal was consistently greater than that of the 5 groups of non-irradiated animals. The difference in the amount of solid residue between irradiated and control rats is shown for one of the 5 groups in Fig. 2A.

It may be noted here that the food consumption of similar rats (above) given the same amount of neutron irradiation was sharply reduced 24 and 48 hours following irradiation. On the 3rd day the food consumption

began to increase and on the 4th day had nearly reached the pre-irradiation level. In order to determine whether or not this recovery of the normal food consumption rate coincided with a return to normal of those gastric functions responsible for the movement of food from the stomach into the intestinal tract, and to determine whether the effect in increasing the solid residue was of long duration, 6 additional groups of 3 rats each were used as follows:

Group	Amount of Irradiation	Fasted
IV	56.4 n	For 24 hours beginning immediately after completion of radiation
V	0	" " " " " " " " "
VI	56.4 n	" " " " 1 day " " " "
VII	0	" " " " 1 day " " " "
VIII	56.4 n	" " " " 2 days " " " "
IX	0	" " " " 2 days " " " "

At the end of the fasting period the rats were killed and the solid residues in the stomachs dried and weighed. The amount of food residues in the stomachs of the irradiated rats (Fig. 2B) was much greater than that in the controls at the end of 24 hours (Groups IV and V) and 48 hours (Groups VI and VII). At the end of 3 days (Groups VIII and IX), however, the amount of solid residue was equal in the stomachs of the irradiated and the control groups. This suggests that the reduced food intake of rats following irradiation is the result of food remaining in the stomach longer than it normally would and that normal consumption is resumed when the stomach has recovered its normal functions.

Residual Fluid Volume. When drinking water was withheld from the animals after irradiation, the amount of fluid in the stomachs of the irradiated animals was only slightly, but consistently, greater than the amount in non-irradiated rats (Fig. 3). However, when the rats had free access to water the amount of fluid in the stomachs of the irradiated rats showed a large and sharp increase over the amount in the stomachs of the controls after the first hour following the test meal (Fig. 3). At the end of $2\frac{1}{2}$ hours the volume in the irradiated animals' stomachs was over twice the amount in the stomachs of the control animals.

pH of the Gastric Juice. The average pH values found in 3 experiments using the gastric fluid of 9 rats for each determination are shown in Fig. 4A. There was, in general, a higher pH in the irradiated groups, although, in the early samples following the test meal, the pH values were lower than those from non-irradiated rats. This indicates that acid secretion in the stomach is impaired by neutron radiation.

Total Acidity. During the first $1\frac{1}{2}$ to 2 hours following the administration of the test meal the total acidity was slightly higher in the stomachs of the irradiated animals than in the controls (Fig. 4B). This is consistent

with a lower pH at this time. After 2 hours the total acidity in the stomachs of the irradiated animals was very much less than that in the control rats until at the end of 5 hours the values were approximately equal. This

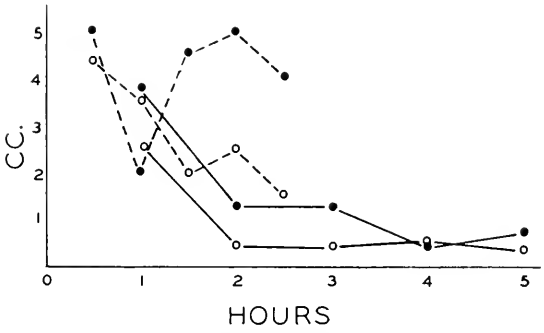


FIG. 3. The effect of 56.4 n on the fluid content of the stomachs of rats. ——— Water withheld. ---------- Water *ad lib.* ● Irradiated. ○ Control.

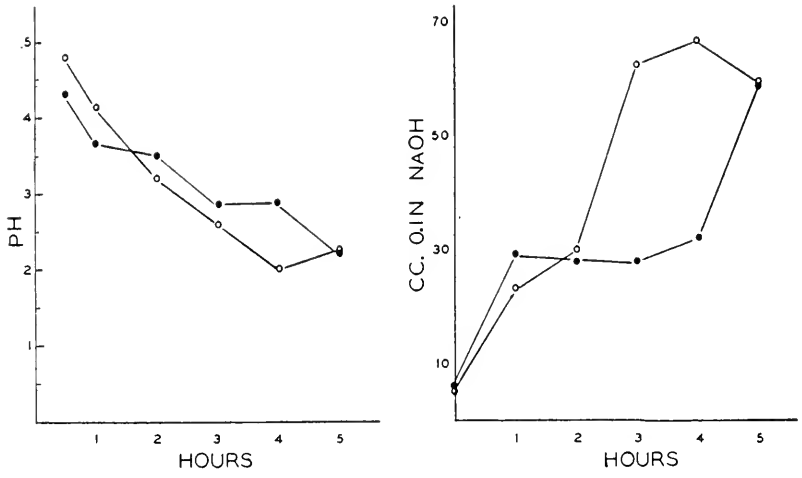


FIG. 4A AND B. The effect of 56.4 n on the pH and on the total acidity of the gastric juice of rats. ● Irradiated. ○ Control.

is consistent with a higher pH of the gastric fluid in the irradiated animals at this period.

Peptic Activity of the Gastric Juice. The peptic activity of the gastric juice of the irradiated rats, determined by the method of Mett (2), was approximately the same as that of the controls during the first $1\frac{1}{2}$ hours

following administration of the test meal. However, after this time the digestive activity of the gastric fluid of the irradiated rats was much less than that of the controls (Fig. 5) until at the end of 4 hours they were approximately the same.

Comment. In normal rats food consumption initiates a series of activities associated with gastric digestion: secretion of digestive juices, active gastric peristalsis, some protein digestion and expulsion of food through the pylorus. In the irradiated rats the food remained longer in the stomach; there was a slightly greater amount of fluid, a reduced total acidity, a higher pH of the fluid and a reduced digestive ability.

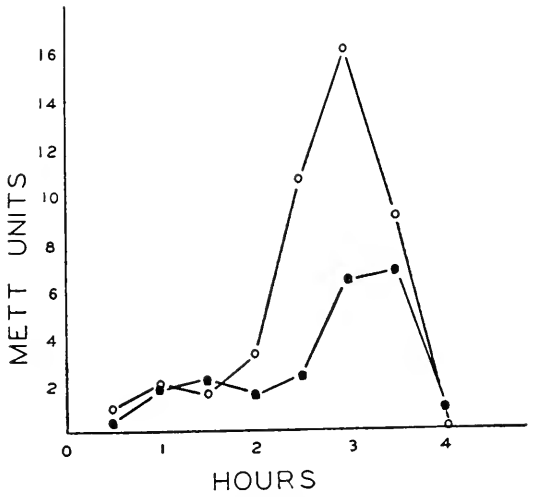


FIG. 5. The effect of 56.4 n on the peptic activity of the gastric juice of rats. ● Irradiated. ○ Control.

The factors initiating these abnormal changes after neutron irradiation are not known. It is conceivable that such factors as reduced muscular activity of the stomach, spasm of the pylorus, and changes in the chemical, enzymatic and nervous systems contribute to, or are responsible for, the delayed expulsion of food from the stomach. This retention then may be responsible for the reduced food intake of irradiated animals.

INFLUENCE OF AGE, WEIGHT AND STATE OF NUTRITION ON RESPONSE TO NEUTRON IRRADIATION

In the course of investigations concerning the effects of neutron radiation on rats, it appeared that older rats withstood exposure to neutrons with less severe effects than younger ones. Further observations concerning this

TABLE I

The Influence of Age, Weight, and State of Nutrition on the Response of Rats to 141 n

Group	Rats	Age	Average Weight	Dietary Treatment (water ad lib.)	Deaths after Irradiation (days)															Total Deaths
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
		days	*grams*																		
Xa	5 M 5 F	58	59	Fox Blox ad lib.			1	2					1	3		1					8
Xb	5 M 5 F	120	265	Fox Blox ad lib.										2							2
XIa	5 M 5 F	86	95	Fox Blox restricted between 26th and 86th days					2		1	2	1	1	1			1			9
XIb	5 M 5 F	86	197	Fox Blox supplemeated with dried milk and yeast between 26th and 86th days								2	1								3
XIc	5 M 5 F	105	197	Fox Blox restricted between 75th and 105th days						1											1
XIIa	5 M 5 F	60	59	Fox Blox; fasted 3 days prior to irradiation				5	1				2								8
XIIb	5 M 5 F	95	165	Fox Blox; fasted 3 days prior to irradiation						1	1	1	3				1			1	8

subject were made by 1) a comparison of the mortality response of older and heavier rats (Group Xa) with that of younger and smaller ones (Group Xb); 2) a comparison of the mortality response of rats, small because of dietary restriction (Group XIa) with that of larger rats of the same age whose diet had not been restricted but supplemented (Group XIb); 3) a comparison of the mortality response of rats of a given weight (Group XIb) with the response of rats of the same weight, but older (Group XIc); 4) a comparison of the mortality response of small young rats (Group XIIa) with that of older and larger ones (Group XIIb), both fasted for 3 days immediately prior to irradiation. All rats had free access to water.

The rats were irradiated (Enns *et al.* (1)) in Box No. 7 with 141.0 n at the approximate rate of 1 n/min.

Small young rats were found to be more radiosensitive than were older and heavier rats (Table I). Rats of the same age, but differing in weight because of dietary restrictions, were found to differ in their sensitivity, the smaller rats being the more sensitive. Rats of the same weight, but differing in age, appeared, on the basis of the limited data, to differ in sensitivity, the younger ones being the more sensitive.

The total mortality was the same for rats of different ages and weights fasted prior to irradiation, but the younger and smaller rats apparently were more sensitive to irradiation than the older and larger ones because they died sooner after irradiation.

The gross clinical changes in the smaller and younger rats after neutron radiation were more pronounced than in the older and heavier animals. In all rats after irradiation, the hair had a dry, coarse, rough appearance. All of the young small rats were smeared and dirty. They were reluctant to move and did so sluggishly, stiffly and with arched back. They were markedly dehydrated and gaunt in appearance, which reflected the greater relative reduction in food intake of the younger, smaller rats than of the older, heavier animals.

These results suggest the advisability, when comparing radiation effects on animals, of taking into account the variable factors of size, age, and nutritional condition.

LACK OF INFLUENCE OF VITAMIN E ON RECOVERY OF TESTES FROM NEUTRON IRRADIATION INJURY

The food intake of rats receiving 56.4 n has been shown to be considerably reduced, thus reducing the amount of vitamins available to the animals. Testicular injury found in rats after neutron irradiation (3) may have been due, to some extent, to lack of vitamin E, or to disturbances in the absorption and utilization of vitamin E.

Four groups of 5 male rats were used: one group, XIV, was fed vitamin

E, another group, XV, was irradiated, the 3rd group, XVI, was fed vitamin E and was irradiated, and the 4th group, XIII, served as a control. The vitamin E supplement was wheat-germ oil (Eli Lilly and Co.) and was given orally in 0.5 cc. amounts every 2nd day beginning on the day that irradiation was completed. The animals were irradiated (1) in Box No. 7. All animals were killed on the 24th day after Groups XV and XVI were irradiated. The moisture content of the testes was determined by weight differences before and after drying at 100°C.

The wet weight of the testes of the irradiated animals was found to be reduced, regardless of vitamin E supplement (Table II). The moisture

TABLE II

The Effect of Vitamin E on Testicular Injury by 56.4 n

Group	No. of Rats	Irradiation	Vitamin E	Average Animal Weight		Days Post-Irradiation	Average Wet Weight of One Testicle
				Initial	Final		
Wheat-Germ Oil Fed by Stomach Tube							
		n		*grams*	*grams*		*grams*
XIII	5	0	no	164	240	—	1.23
XIV	5	0	yes	157	251	—	1.65
XV	5	56.4	no	163	205	24	0.76
XVI	5	56.4	yes	164	195	24	0.67
α-Tocopherol Injected Intramuscularly							
XIII	5	0	no	162.2	256.2	—	1.55
XIV	5	0	yes	163.0	254.2	—	1.57
XV	5	56.4	no	161.3	225.4	24	0.91
XVI	5	56.4	yes	162.0	220.0	24	0.96

content of the testes was the same in all groups. Histopathological examination of the testes of the irradiated animals showed no difference in the amount of injury in those that were given the vitamin E supplement and those which did not receive it. The supplement of wheat-germ oil had no effect in preventing loss of weight of the rats following irradiation. The experiment was repeated with the same number of animals, and the results were similar.

These results do not preclude the possibility that pathological conditions due to vitamin E deficiency and those produced by neutron radiation are similar, since, following irradiation, absorption of vitamin E from the intestinal tract or the actual utilization of the vitamin by cells may be disturbed.

In order to eliminate the possibility of lack of effect because of nonabsorption from the intestinal tract, the vitamin E, in the form of α-toco-

pherol, was injected intramuscularly. Four groups of 5 male rats were used as in the previous experiment, with the exception that the vitamin E was injected intramuscularly. Treatment was continued for 24 days. The dose was 0.1 cc., containing 1 mg. α-tocopherol, daily.

α-Tocopherol, given intramuscularly (Table II), also appeared to have no effect in alleviating the testicular injury or loss of body weight following irradiation.

Since vitamin E given orally as wheat-germ oil or given intramuscularly as α-tocopherol apparently had no influence on the degree or nature of injury produced by neutron radiation in the testes of rats, it would appear that the injury caused by neutron irradiation differs from that caused by vitamin E deficiency (4). In both cases the injuries are progressive and are reversible within limits. It may be that the ability of the cells of the testes of the irradiated rat to metabolize vitamin E is impaired or that other injuries to the cell are limiting factors.

SUMMARY

1. Loss in weight after a single dose of 56.4 n was accompanied by a reduced food consumption in rats. When the food intake of non-irradiated rats was restricted to the amount consumed by the irradiated ones their weight was reduced a comparable amount. The reduced food consumption by rats after a single dose of 56.4 n appeared to be responsible for the loss in body weight.

2. Irradiation of the rat with 56.4 n was found to disturb gastric digestion, as shown by a decreased total acidity, higher pH of the gastric fluid, reduced peptic activity and prolonged food retention in the stomach.

3. When rats were irradiated with 141 n, small young rats were found to be more sensitive than older and larger ones. Of rats of equal age, but differing in weight because of dietary restriction, the smaller were more sensitive than the larger. Young and small rats apparently were more sensitive than older and larger ones when both were fasted prior to irradiation.

4. The administration of vitamin E, either orally or intramuscularly, did not appear to prevent testicular injury following neutron irradiation.

REFERENCES

(1) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(2) Mett, in Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry", 12th Ed., The Blakiston Company, Philadelphia (1947), p. 347.
(3) Ely, J. O., Ross, M. H., and Gay, D. M., Chapter 20.
(4) Mason, K. E., *J. Exptl. Zool.*, **55**, 101 (1930).

Chapter 18

EFFECTS OF NEUTRONS ON WHITE BLOOD CELL COUNT AND BLOOD SEDIMENTATION RATE OF RATS

By M. H. ROSS and J. O. ELY

Changes in the hematopoietic system frequently indicate the condition of the organism as a whole. Alterations in the peripheral white blood cell count often occur in bacterial and parasitic infections, inflammatory processes, in leukemia, after the absorption of certain drugs and poisons and after penetrating radiations. Other diverse conditions such as digestion, emotional disturbances (1), hormone imbalance (2), reduction in food intake (3) and reduction in required food elements have been shown to influence the production of blood cells or their discharge into the peripheral blood (4). The number and kind of white blood cells affected are often indicative of the severity of the condition.

Alterations in the fluid portion of the blood, indicated by increased rate of sedimentation and by electrophoresis may be effected by disturbances (5) in fat metabolism, cirrhosis of the liver, by hemorrhage, fever, by foreign protein reactions and by neutron irradiation (6). Shedlovsky and Scudder (7) and Rogatz (8) stated that an increase in the rate of blood sedimentation was always demonstrable when any considerable tissue destruction had occurred. Considerable tissue damage was found to be present in certain radiosensitive tissues and organs after irradiation (9). Changes in the blood sedimentation rate (10) therefore offer another criterion of the severity of the condition.

Lawrence *et al.* (11, 12) found that, in mice, leukopenia, lymphopenia and neutropenia follow neutron irradiation. These blood changes are similar to those following X-radiation. Warren and Dunlap (13) have reviewed the cellular changes and the changes in the plasma and serum following penetrating radiations. The changes in the white blood cells following X-ray and neutron irradiation are reported to be similar. The lymphocyte was shown to be particularly sensitive to all forms of penetrating radiation. Neutrophils were found to be radiosensitive but not to such a marked degree. Following X-radiation numbers of degenerated neutrophils have been reported.

The effects of single and of multiple doses of neutron radiation on the white blood cell count of rats have been studied. The effects of a single dose of neutron radiation on the blood sedimentation rate was also investigated.

Changes in Number of White Blood Cells after Neutron Irradiation. Three groups of 20 male rats, each rat weighing approximately 200 grams, were irradiated (Enns *et al.* (14)) in Box No. 7 with 11.3, 56.4 and 113 n respectively. The rate of administration of the doses was approximately 1 n per minute. The dose of 113 n was given in equal parts on two successive days.

Blood was taken from the tail. Pre-irradiation counts were made on all animals. After irradiation, counts were made daily at the same hour until recovery was practically complete, then twice weekly.

11.3 n: Twenty-seven hours after completion of irradiation there was a marked decrease in the total white blood cell count. The minimum value was reached between the 4th and 7th days after irradiation. The return to normal was rapid and full recovery had occurred by the 22nd day (Fig. 1A).

A temporary lymphopenia reached a maximum on the 2nd day after completion of irradiation (Fig. 1B). Return to the pre-irradiation value was rapid and was reached approximately on the 7th day. The reduction in the absolute number of lymphocytes reached its maximum on the 2nd day. This depression occurred 2 to 5 days sooner than the maximum leukopenia or the maximum absolute reduction of neutrophils. However, return to normal of the absolute lymphocyte count paralleled that of the leukocyte count.

The relative neutrophils increased until the second day (Fig. 1B) but, the absolute neutrophil count was depressed (Fig. 1A) until the 7th day and then returned to the pre-irradiation level about the 9th day.

56.4 n: There was a profound drop in the total white blood cell count in the first 24 hours after irradiation. The maximum depression was reached on the 3rd day (Fig. 1C). After this there was a rise in the total white blood cell count so that about the 32nd day the count approximated the pre-irradiation level. The changing values for the relative number of lymphocytes and neutrophils are shown in Fig. 1D. The greatest reduction in the relative number of lymphocytes and the greatest increase in the relative number of neutrophils were reached on the 2nd day. The differential count had almost reached the pre-irradiation values 8 days after irradiation and fully reached them by the 32nd day. The absolute number of lymphocytes reached its maximum depression at approximately the same time as that of the total number of leukocytes and a short time before the maximum depression of the absolute number of neutrophils. Return to pre-irradiation values of the lymphocyte count paralleled that of the total white blood cell count. The relative neutrophilia had returned to pre-irradiation values on the 5th day (Fig. 1C), but the absolute neutropenia

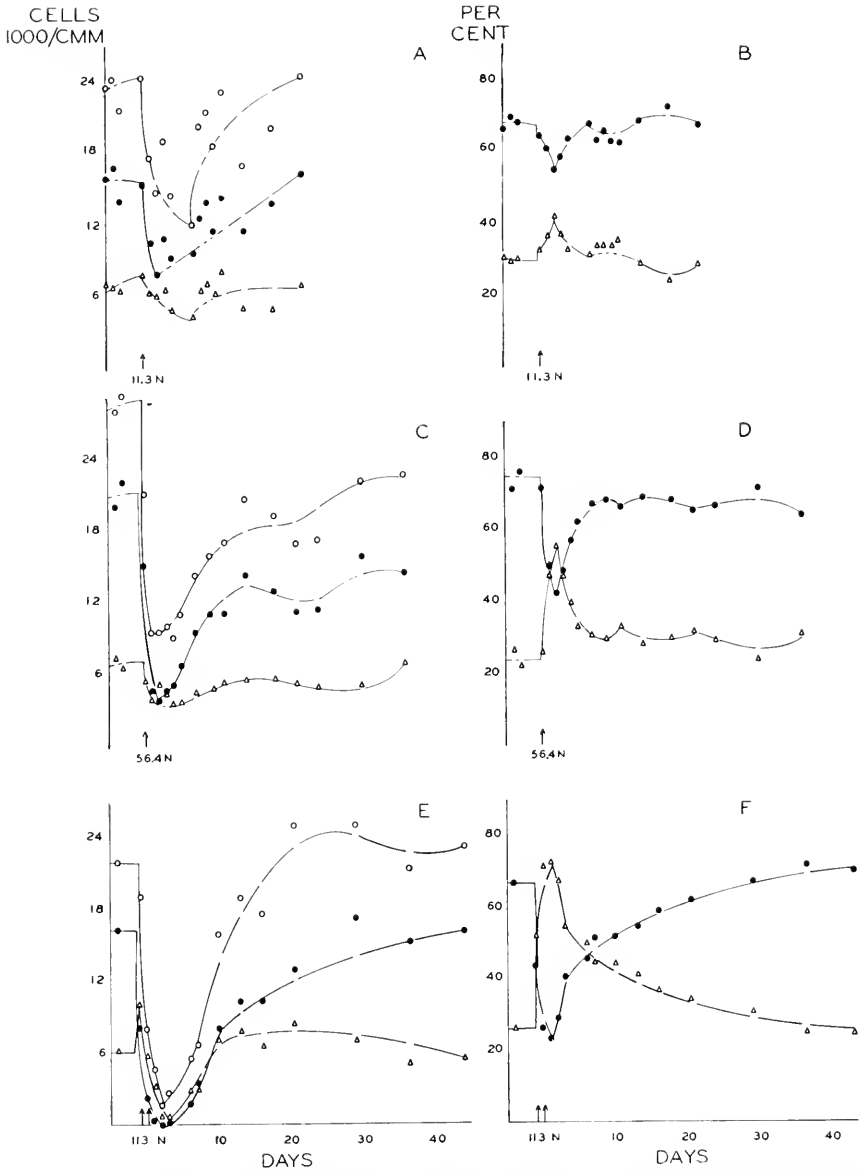


FIG. 1. Effect of different doses of neutron radiation on the white blood cell count of rats. A, C, E—total counts: ○ Total leukocyte count; ● Absolute lymphocyte count; △ Absolute neutrophil count. B, D, F—differential counts: ● Lymphocytes; △ Neutrophils.

reached its maximum depression at that time (Fig. 1D). The blood changes occurring after 11.3 n and 56.4 n differed in time and intensity only.

113 n: Immediately after irradiation there was an enormous drop in the total leukocyte count (Fig. 1E) which was lowest on the 2nd day. The count had risen above the pre-irradiation level by the 28th day, and returned to pre-irradiation values after the 40th day. The changes in the differential values for the lymphocytes and neutrophils are shown in Fig. 1F, with a relative lymphopenia and neutrophilia indicated. Both the absolute number of neutrophils and the absolute number of lymphocytes were lowest on the 2nd day (Fig. 1E). The lymphocyte count paralleled that of the white blood cell count.

TABLE I

Effects of 56.4 n and 113 n, Given in Single and Divided Doses, on the White Blood Cells of Rats

No. of Rats	Dose per Day	No. of Doses	Maximum Reduction Total White Cell Count	Greatest Reduction Abs. Lymphocyte Count	Greatest Reduction Abs. Neutrophil Count
	n		%	%	%
20	56.4	1	74.0	82.1	43.7
10	28.2	2	76.6	88.1	52.9
10	11.3	5	74.9	86.8	40.8
10	5.6	10	74.3	79.2	64.1
20	113	1	87.5	90.7	75.5
20	56.4	2	89.8	95.4	74.5

As the total leukocyte count approached pre-irradiation values, numerous abnormal cells were found in the blood smears. Some of the lymphocytes were very small, some showed cytoplasmic vacuolization. Neutrophils containing 8 or more segments in the nucleus were seen along with occasional degenerated forms. There were a few small vacuolated basket forms. Abnormal unclassified cells which were present shortly before recovery occurred twice as frequently in this group as in the group given 56.4 n, and 10 times as frequently as in non-irradiated rats.

Eosinophils, basophils, monocytes, myelocytes, blast forms and plasma cells were so few and variable in number that no conclusions could be drawn concerning the effect of neutrons upon them. Besides the abnormal cells, the total number of primitive cells increased after irradiation.

Comparison of Effects of Single and of Divided Doses. One group of rats was given 56.4 n in a single dose, the 2nd group was given 28.2 n on each of 2 successive days, the 3rd group was given 11.3 n on each of 5 successive days, the 4th group was given 5.6 n on each of 10 successive days. The total dose was approximately 56.4 n in each case.

Two additional groups of rats received 113 n: one group received it in one dose, the other received 56.4 n on each of 2 successive days.

The changes in the total white blood cell and absolute lymphocyte counts were found to be approximately the same, whether single or divided doses were administered (Table I).

Neutron Dose and the Degree of Depression. Five groups of rats, each rat weighing approximately 200 grams, were given 11.3, 28.2, 56.4, 84.6 and 113 n respectively. Each group consisted of 20 rats, with the exception of the group receiving 84.6 n, which had 10 rats. Blood counts were made daily until definite signs of recovery appeared.

With all doses of neutron radiation given, the decrease in numbers of cells began in the first 24 hours. As the dose was increased the initial decrease became greater. The greatest depression in the number of leukocytes and absolute number of neutrophils occurred at different intervals after irradiation, while the lowest number of lymphocytes always occurred on the 2nd day. The period of time for return to the pre-irradiation blood

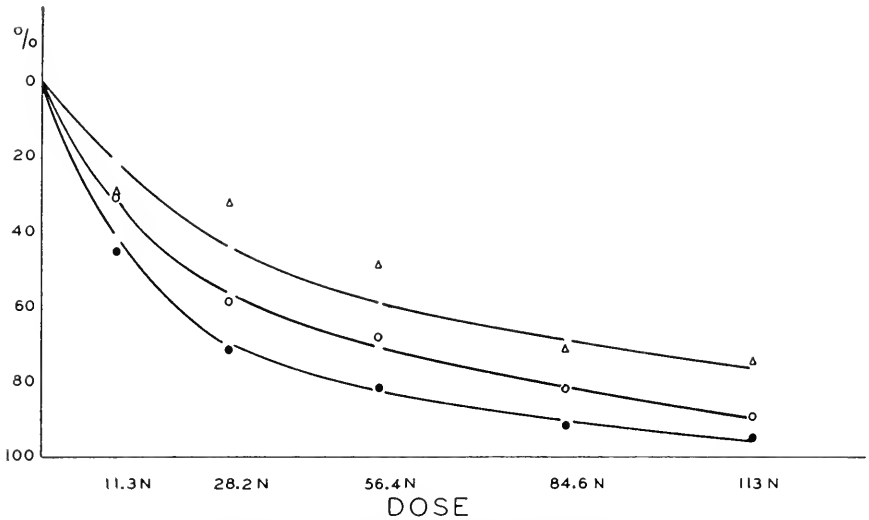


FIG. 2. Lowest white blood cell count, expressed as per cent. of pre-irradiation count, at different dosage levels. ● Absolute lymphocyte count; ○ Total leukocyte count; △ Absolute neutrophil count.

values was found to vary with the dose. The greater the dose, the greater was the depression and the longer the duration of the depression.

The lowest number of leukocytes occurred on the 7th, 4th, and 2nd days for 11.3 n, 56.4 n and 113 n respectively while the lowest absolute number of

neutrophils occurred on the 7th, 4th and 3rd days respectively. The length of time after irradiation required for the lowest number of leukocytes and neutrophils to be reached was found to be inversely proportional to the size of the dose.

The lowest white blood cell count for each dose, expressed as percentage of pre-irradiation count, is shown in Fig. 2. The rate of the depression decreased as the dose was increased.

Influence of Neutrons on the Blood Sedimentation Rate. Ten male rats weighing about 300 grams each were divided into two groups of five. Prior to irradiation the blood sedimentation rate of each animal was determined by the Landau-Adams microsedimentation method (15). Blood was obtained from the tail. The rats of one group were given 56.4 n in Box No. 7 and the other group served as a control. The blood sedimentation rates of the animals of both groups were determined at five intervals during the succeeding four weeks.

An increase in the blood sedimentation rate of the irradiated rats was found one day after completion of irradiation. Thereafter there was a gradual increase until the eighth day, when the blood sedimentation rate of the irradiated animals had increased 100 per cent over the pre-irradiation rate and was 29 per cent greater than that of the non-irradiated controls at that time. After the eighth day the rate decreased gradually until at the end of four weeks the normal pre-irradiation value was reached. The observations were then discontinued.

DISCUSSION

The changes in numbers and kind of white blood cells after neutron irradiation are similar to the reported blood changes after X-radiation. This similarity is particularly emphasized in the sensitivity of the lymphocyte. There is an almost immediate and a profound response: a reduction in numbers of lymphocytes in the peripheral blood stream and an immediate cessation of cellular mitotic activity in lymphoidal organs. The reversal of the lymphocyte-neutrophil ratio illustrates the greater radiosensitivity of lymphocytes over that of neutrophils to penetrating radiation. Considering the degree of lymphopenia produced, the recovery of the lymphocyte is extremely rapid. While neutrophils were also affected, the counts never decreased in the same proportion as the lymphocytes. Neutrophils seem to be more resistant to radiation than lymphocytes. This serves to illustrate which cells of the blood are more responsive to irradiation. In both X-ray and neutron irradiation there were considerable numbers of degenerated neutrophils.

It is difficult to interpret the changes in the blood count after irradiation as a direct or an indirect effect. Not all of the changes are necessarily

due to direct effects of the irradiation. Food consumption in rats after neutron irradiation is materially decreased and a reduced food consumption indirectly depresses the numbers of white blood cells in the peripheral blood. Irradiation also may affect the function of the adrenals and the pituitary and through a hormonal imbalance indirectly affect the blood count.

No measurable recovery of the animals was evident during the short intervals between irradiation periods, so far as the effect on the white blood cell system was concerned. It would be interesting to know how long the intervals between the application of the fractional doses of irradiation could be extended before a difference in the effects of a total and a divided dose could be detected.

The changes in sedimentation may be due to a direct action of the irradiation on the blood or they may be due to an indirect action.

It is possible that blood sedimentation is influenced by substances released from injured tissues after neutron irradiation. Disturbances in metabolism following irradiation may result in an alteration in the components of the plasma, thus influencing the rate of blood sedimentation.

SUMMARY

Leukopenia, lymphopenia and neutropenia were found in rats following doses of 11.3 to 113 n. The maximum decrease and rate of decrease in number of white blood cells were greater with increasing amounts of neutron irradiation. The rate of decrease following any individual dose was greatest during the first 24 hours. The greater the dose, the longer was the period of time required for complete recovery. The lymphocyte appeared to be more radiosensitive than the neutrophil.

There was no significant difference between the effect on the white blood cell count, absolute lymphocyte count or absolute neutrophil count of rats when the total dose of 56.4 n or 113 n was given in one day and that when interrupted doses were given over several days.

An increased blood sedimentation rate was found in rats that had received 56.4 n. The maximum increase occurred eight days after irradiation. Thereafter the sedimentation rate decreased slowly until it reached pre-irradiation values.

REFERENCES

(1) Farris, E. J., *Am. J. Anat.*, **63**, 325 (1938).
(2) Doughfirty, T. F., and White, A., *Am. J. Anat.*, **77**, 81 (1945).
(3) Ershoff, B. H., and Adams, A. D., Jr., *Proc. Soc. Exptl. Biol. Med.*, **62**, 154 (1946).
(4) Ely, J. O., and Ross, M. H., Chapter 17.
(5) Cohn, E. J., *Chem. Rev.*, **28**, 395 (1941).
(6) Sanigar, E. B., Chapter 15.

(7) Shedlovsky, T., and Scudder, J., *J. Exptl. Med.*, **75,** 119 (1942).
(8) Rogatz, J. O., *J. Lab. Clin. Med.*, **28,** 1842 (1943).
(9) Ely, J. O., Ross, M. H., and Gay, D. M., Chapter 20.
(10) Ross, M. H., and Ely, J. O., Chapter 19.
(11) Lawrence, J. H., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22,** 124 (1936).
(12) Lawrence, J. H., Aebersold, P. C., and Lawrence, E. O., *Proc. Natl. Acad. Sci.*, **22,** 543 (1936).
(13) Warren, S., and Dunlap, C. E., *Arch. Path.*, **34,** 562 (1942).
(14) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(15) Landau, A., *Am. J. Diseases Children*, **56,** 694 (1933).

CHAPTER 19

EFFECTS OF LARGE DOSES OF NEUTRONS ON DOGS

BY M. H. ROSS AND J. O. ELY

Reports on the severe clinical and chemical effects of an instantaneous massive dose of all of the known types of penetrating radiation on human beings (1) indicated the importance of further study of the changes induced in living tissue. Laboratory studies on the biological effect of neutron radiation indicated that the hematopoietic (2) and spermatogenic tissues (3) and the tissues of the gastrointestinal tract (3) are profoundly affected. In order to study the clinical, pathological, hematological and blood chemical changes associated with large doses of neutron radiation, four dogs, available from other work, were used in the following investigations.

Two adult male and 2 adult female dogs of mixed breeds were given a total of 400 n during 4 consecutive days: 115 n on the 1st, 2nd and 3rd days and 55 n on the 4th day. The rate of administration, in Box No. 4, was approximately 18 n per hour (4).

The dogs had free access to water at all times. They were fed once a day and allowed at that time all the food (Friskies) they desired. The average daily food consumption for the 21 day pre-irradiation period was 8 oz. per dog. During the post-irradiation period the food was available for 4 to 5 hours each day.

Clinical Effects. No food was consumed after completion of irradiation. During the 4 days required to administer the irradiation, the average daily food intake was 2 oz. per dog.

The average weight of the dogs decreased rapidly following irradiation. Water intake was decreased; the dogs became dehydrated, cachectic, phlegmatic and weak and, shortly before death, comatose. Two of the 4 dogs had foul breath. Both of these dogs had gray, necrotic ulcers in the mouth. The skin was dry, the hair coarse, rough and lustreless, and the skin and hair had a peculiar odor. Before death 2 of the dogs passed thick, mucoid, bloody feces. One dog vomited 1 day before death. One dog died 6 days, one $7\frac{1}{2}$ days, and one 8 days following completion of irradiation. The other one was killed on the 7th day.

No blood pressure measurements were made, but great difficulty was experienced in obtaining blood from the radial vein of the forearm after completion of irradiation, indicating a reduced venous blood pressure. A reduced blood pressure was also indicated by a weak and rapid heart action.

Electrocardiograms indicated progressive myocardial damage, probably on an anoxemic basis.

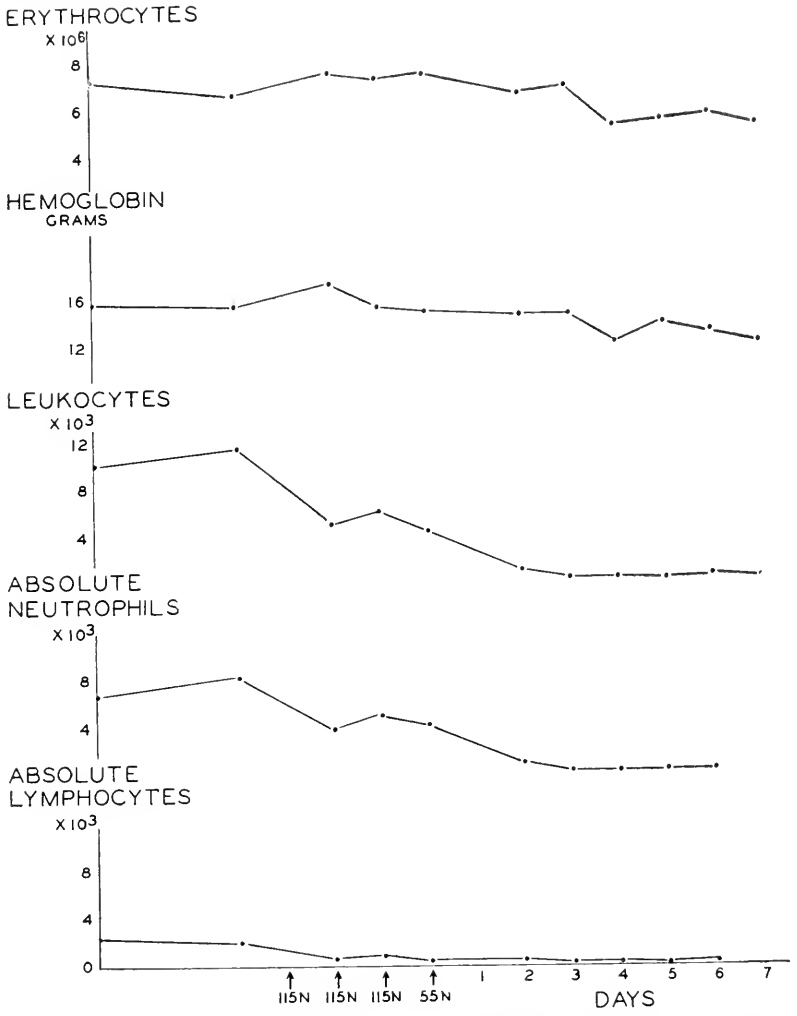


FIG. 1. Effect of 400 r on the leukocyte counts, red blood cell counts and hemoglobin of dogs.

Hematological Effects. All dogs reacted essentially the same. The red blood cell and hemoglobin values declined an average of 21.5 per cent. and 17 per cent. respectively during the interval between irradiation and death (Fig. 1). The average hematocrit values declined (Table I). The white

blood cell counts, the absolute lymphocyte counts and the absolute neutrophil counts began to decline immediately after the first day of irradiation (Fig. 1). The decline in the neutrophil count consistently paralleled the total white blood cell count. The maximum reduction in blood cell values

TABLE I

Hematocrit Values and Specific Gravity of Whole Blood and Plasma

Days after Irradiation	No. of Dogs	Hematocrit	Specific Gravity	
			Whole Blood	Plasma
		average	*average*	*average*
(Pre-irradiation)	4	51.75	1.0616	1.0225
3	4	46.25	1.0559	1.0210
6	4	43.64	1.0542	1.0236
8	1	37.50	1.0553	1.0232

TABLE II

Blood Chemistry of Neutron-irradiated Dogs. Average Values

Days after Irradiation	No. of Dogs	Glucose (Blood)	Chlorides as NaCl (Blood)	Calcium (Serum)	Inorganic Phosphorus (Plasma)	Total Plasma Nitrogen	Non-protein Nitrogen of Plasma
		mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./ml.*	*mg./ml.*
(Pre-irradiation)	4	94.3	502	10.4	1.99	10.12	0.300
3	4	106.5	459	13.3	2.38	10.01	0.233
6	4	122.0	468	12.5	2.85	11.89	0.247
8	1	162.0	500			12.32	0.205

occurred on the 5th day after completion of irradiation and was, for white blood cells 98.9 per cent., for absolute neutrophils 99.2 per cent. and for absolute lymphocytes 97.9 per cent. The eosinophil values declined 96 per cent. Reticulocytes disappeared completely from the blood. Platelets were reduced 72 per cent.

No definite change was noted in the percentage of basophils, nor in the degree of poikilocytosis, anisocytosis, or basophilia of the red cells. Erythroblasts and plasma cells were seen only in the blood of dog #2 after irradiation.

The profound changes in the blood sedimentation rate following irradiation are shown in Fig. 2. Blood taken 3 days after completion of irradiation showed an increase in sedimentation rate, for a 24 hour sedimentation period, of 683 per cent. On the 6th day, the increase was 1,860 per cent.

The specific gravity of the blood, determined by the method of Barbour and Hamilton (5), decreased progressively after irradiation. The specific

gravity of the plasma decreased slightly at first, then increased slightly above the pre-irradiation level (Table I).

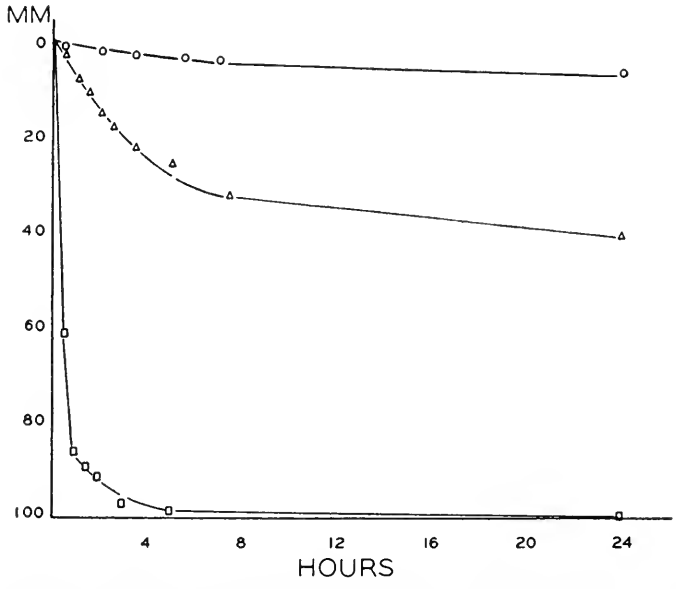


FIG. 2. Effect of 400 n on the blood sedimentation rate of dogs. ○ Pre-irradiation. △ 3 days after irradiation. □ 6 days after irradiation.

No effect on the fragility of the erythrocytes was found.

Blood Chemistry. Animals were fasted for 24 hours before samples of blood were taken. Determinations of the blood glucose (6), blood chloride (7), serum calcium (8), inorganic phosphorus (9), plasma total nitrogen and plasma non-protein nitrogen (10, 11) were made prior to irradiation and 3, 6 and 8 days after completion of irradiation.

Chloride values remained relatively constant (Table II). Glucose concentrations increased slightly but consistently. From dog #1, the last sample of blood was taken only 10 or 15 minutes before death. The glucose concentration for this sample was 72 per cent. higher than the initial average for the 4 dogs.

The serum calcium increase averaged 20 per cent. in the six days following completion of irradiation. The inorganic phosphorus increased approximately 43 per cent. Total nitrogen of the plasma increased approximately 17 per cent. in six days, while the average decrease of non-protein nitrogen was 17.7 per cent.

Gross Pathological Findings. See table on page 164.

Histopathological Findings (By Douglas M. Gray). See table on page 166.

GROSS PATHOLOGICAL FINDINGS*

	Dog #1 Male	Dog #2 Male	Dog #3 Female	Dog #4 Female
Subcutaneous tissues	Hemorrhage in legs, profound in right hind and left front. Hemorrhage at rib ends and near sternum.	—	—	Hemorrhage and edema of hocks.
Oral cavity	Mucosa swollen; gray necrotic ulcers on labial portions of gums.	Small ulcers on gums.	Confluent gray necrotic areas on gums.	—
Lungs	Emphysematous blebs; cooked appearance.	—	Emphysematous blebs; left posterior lobe completely hemorrhagic; hepatinization; other lobes diffusely but profoundly hemorrhagic. Edematous fluid.	—
Heart	—	—	Small hemorrhages in region of sino-auricular node; diffuse hemorrhages in apex.	—
Stomach	Yellow fluid; no food. Petechial hemorrhages scattered throughout mucosa.	Yellow fluid; no food.	Yellow fluid; no food. Small erosions in fundic portions.	Yellow fluid; no food.
Intestine	No ingesta. Scattered areas of petechial hemorrhage. One large bloody area, $\frac{1}{2}$ inch in diameter in colon.	No ingesta. Areas of petechial hemorrhage.	No ingesta.	No ingesta. Areas of petechial hemorrhage in colon.

Spleen	Hard; dry; shriveled surface. Trabeculae prominent on cut section.	Rough; black; capsule adherent. Trabeculae prominent on cut section.	Small; pale pink; shriveled surface. Trabeculae prominent on cut section.	Small; red; shrunken. Trabeculae prominent on cut section.
Kidney	—	Pitted on surface.	Cortical hemorrhage extending into inner zone of medulla.	Capsule adherent.
Urinary bladder	—	Hemorrhage into wall.	—	—
Testes	Hemorrhage in epididymis.	Hemorrhage in epididymis, scrotum and under capsule.	(Female)	(Female)
Liver	—	—	—	Slightly friable.
Lymph nodes	Small, completely hemorrhagic.	Small, completely hemorrhagic.	Small, completely hemorrhagic.	Small, completely hemorrhagic.
Femoral bone marrow	—	Yellow and watery.	—	—

* No gross pathological changes were found in the pancreas, thyroid, adrenals or ovaries. The brain and the pituitary gland were not examined.

HISTOPATHOLOGICAL FINDINGS†

	Dog #1 Male	Dog #2 Male	Dog #3 Female	Dog #4 Female
Lung	—	Some alveoli contained albuminous fluid.	A few alveoli contained small amounts of edema fluid.	—
Spleen	Follicles absent. Sinuses collapsed. Small masses of blood pigment frequent in monocytes.	Follicles absent. Congested.	Small sinuses collapsed. Malpighian corpuscles absent.	Shrunken. Sinuses collapsed except in central portion. Malpighian corpuscles inconspicuous.
Liver	Cells uniformly small in size.	Sinuses around hepatic vein distended. Adjacent cords of liver cells compressed. Passive congestion.	—	—
Stomach	Subepithelial layer contained no lymphocytes.	Mucosa thin. Lymphoid tissue absent.	Mucosa thin.	Mucosa thin. Lymphoid tissue sparse. Rather inactive mucous production.
Intestine	Subepithelial lymphoid tissue absent.	Lymphoid tissue absent.	Mucosa thin. Lymphoid tissue absent. (Female)	Mucosa thin. Lymphoid tissue absent. (Female)
Testicle	Acute degeneration characterized by disruption of layer of germinal epithelium. Cells appeared detached, pyenotic and separated with vacuoles. No active mitosis but morphologically normal sperm seen.	Acute degeneration with separation of many cells. Degrees of shrinkage. Active spermatogenesis absent.		

Seminal vesicles	Large number of sperm. Hemorrhage into surrounding soft tissues.	—	(Female)	(Female)
Lymph nodes	Congested. Small amounts of fresh blood in sinuses.	Recent hemorrhage.	Follicles absent. Sinuses contained edema fluid. Numerous monocytes filled with yellow brown material, probably blood pigment.	Some sinuses contained edema fluid. Germinal centers inconspicuous, difficult to identify.
Striated muscle			Atrophy of some muscle fibers; some infiltration of fat tissue.	—
Gums	Contained bacterial masses, but no polymorphonuclear exudate.	Small ulcer. Underlying tissues homogeneous, indifferently staining mass. No cellular infiltration or exudate.	Homogeneous mass. Clumps of bacteria, remainder unrecognizable.	—
Skin	—	—	Epidermis thin, atrophied, consisting in places of single layer cuboidal basement cells covered with keratinized cells. Hair follicles atrophied.	—

† No histopathological changes were found in the pancreas, adrenals, heart, thyroid or ovaries. The brain and pituitary gland were not examined.

DISCUSSION

Death of the dogs following 400 n cannot be attributed to any particular change resulting from irradiation, nor can the changes be designated as primary or secondary.

The slight differences in the pattern of depression of the blood elements from that in rats (12) and in rabbits (13) may be accounted for by the fact that the dogs received greater amounts of radiation.

The relatively small difference between the decrease in the hematocrit and red blood cell values does not indicate a change in the size of the erythrocyte.

The most marked change was in the blood sedimentation rate which increased rapidly as the animals approached death. Preliminary work indicates that changes in the fluid portion of the blood may be largely responsible for the increased blood sedimentation rate found in these dogs and in rats (12). This contention is supported by electrophoresis studies (14) which have shown changes in the plasma components.

The second greatest effect observed in the dogs was hemorrhages in the lymph glands, heart, epididymis and subcutaneous tissues. This indicates a severe effect on the vascular system, either directly or indirectly.

The increased serum calcium following irradiation may indicate decalcification of the bones. An increase in calcium excretion caused by fasting (15) indicates some dissolution of bony structures. Whether the change in serum calcium was due entirely to fasting is not known, but it may be, since food was refused by the dogs during the post-irradiation period, probably because of radiation sickness.

The increase in inorganic phosphorus following irradiation is consistent with increased phosphatase values for these dogs (16).

SUMMARY

The administration of 400 n to dogs was followed by severe clinical symptoms, such as loss of appetite, loss of weight, subcutaneous hemorrhage and edema, ulceration of the gums, lethargy and cachexia, coarseness of hair and skin, and an impaired heart action.

Hematological changes, such as leukopenia, neutropenia, lymphopenia, occurred along with a reduction of red blood cells, hemoglobin, reticulocytes and blood platelets. The rate of blood sedimentation increased.

Serum calcium, inorganic phosphorus and the total nitrogen content of the blood increased; the non-protein nitrogen content of the plasma decreased. Blood chloride values remained relatively constant.

Histopathological changes found were hemorrhages, destruction of lymphoid tissue and injury to the testes.

REFERENCES

(1) Timmes, J. J., *U. S. Naval Med. Bull.*, **46**, 219 (1946).
(2) Lawrence, J. H., and Lawrence, E. O., *Natl. Acad. Sci.*, **22**, 124 (1936).
(3) Yamashita, H., *Gann*, **31**, 629, German Abstract, 654 (1937).
(4) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(5) Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, **69**, 625 (1926).
(6) Hawk, P. B., Oser, B. L., and Summerson, W. H., "Practical Physiological Chemistry", 12th Ed., The Blakiston Company, Philadelphia (1947), p. 528.
(7) Dean, R. B., and Fishman, M. M., *J. Biol. Chem.*, **140**, 807 (1941).
(8) Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, **63**, 461 (1925).
(9) Gomori, G., *J. Lab. Clin. Med.*, **27**, 955 (1942).
(10) Ma, T. S., and Zuazaga, T., *Ind. Eng. Chem., Anal. Ed.*, **14**, 280 (1942).
(11) Leitch, J. L., and Wells, L. A., *J. Franklin Inst.*, **241**, 73 (1946).
(12) Ross, M. H., and Ely, J. O. Chapter 18.
(13) Sanigar, E. B., Miller, G. L., and Maddox, M. N., Chapter 14.
(14) Sanigar, E. B., Chapter 15.
(15) Best, C. H., and Taylor, N. B., "The Physiological Basis of Medical Practice", 4th Ed., p. 608, The Williams and Wilkins Co., Baltimore (1945).
(16) Reinhart, F. E., Chapter 8.

Chapter 20

CHANGES PRODUCED IN TESTES, SPLEEN, BONE MARROW, LIVER AND KIDNEYS OF RATS BY NEUTRON RADIATION

By J. O. ELY, M. H. ROSS, and DOUGLAS M. GAY

Little has been reported in the literature concerning the histological changes produced in tissues by neutron radiation although many reports have demonstrated the intense biological effects of this type of radiation.

Lawrence and Tennant (1) reported on the histological changes produced in various organs of mice 1–5 days after lethal doses of neutron radiation. They found no qualitative difference between the injuries produced by neutron and X-radiation.

Yamashita (2, 3) described effects of neutron radiation on several organs of immature rats at intervals up to 4 weeks after irradiation.

This report is an account of some studies on the effects of neutron radiation on several organs of rats observed over comparatively long periods of time. Effects on testes and spleen received greatest attention; studies of effects on liver and kidneys were coincidental; those on bone marrow are incomplete, but the available results are included.

EXPERIMENTAL

Testes

A. *Changes in Weight.* Four groups (Nos. 2–5 in Table 1) of male rats were given 17.5, 32.5, 47.5 and 62.5 n respectively (Enns *et al.* (4)) in Box No. 7, the total dose being given in one day. Group 1 served as a control group. Thirty-five days later the animals were killed. One testicle from each rat was used for histological examination; the other was weighed and the moisture content determined. Six other groups of rats (Nos. 7–10, 12 and 13) were given 56.4, 56.4, 60, 120, 180 and 240 n respectively. The 56.4 n doses were administered in Box No. 7, the other doses in Box No. 4. Groups 6 and 11 served as controls.

The testes of the rats from groups 2–5 were much reduced in weight, but no significant difference was found in their moisture content. There was a marked decrease in testicle weight in relation to body weight in groups 12 and 13 in 22 to 24 days after the administration of 56.4 n and in group 7 after 60 n. At the higher irradiation levels (120 to 240 n) the animals died, presumably before any change in weight could occur, since

atrophy and shrinkage of the seminiferous tubules appear some time after irradiation, the length of the lag period depending on the dose.

B. Histopathological Changes. Four groups of 20 or more male rats each were exposed to 11.3, 56.4, 113 and 355 n units of radiation respectively in Box No. 7. Immediately after irradiation and at intervals up to

TABLE I

Effect of Neutron Irradiation on the Weight of Rat Testes, Spleen and Liver

Group	No. of Rats	Initial Weight of Rats (Average)	Radiation			After Irradiation	Final Weight of Rats (Average)	Wet Weight One Testicle (Average)	Rat Weight / Testes	Wet Weight Spleen (Average)	Rat Weight / Spleen	Rat Weight / Liver
		grams	*n*		*Box* #	*days*	*grams*	*grams*	*ratio*	*grams*	*ratio*	*ratio*
1	12	220	0			—	261	1.93	136	1.21	216	
2	6	211	17.5	(1 day)	#7	35	238	1.21	197	0.91	262	
3	6	215	32.5	(1 day)	#7	35	239	0.85	281	1.05	230	
4	6	217	47.5	(1 day)	#7	35	248	0.81	306	0.96	258	
5	6	220	62.5	(1 day)	#7	35	239	0.88	272	0.99	242	
6	11	203	0			—	231	1.47	158	1.25	185	20.1
7	6	208	60	(1 day)	#4	22	193	0.81	238	1.32	147	20.0
8	6	209	120	(2 days)	#4	10.5	157	0.97	162	0.68	232	21.8
9	6	203	180	(3 days)	#4	4.3 (died)	149	1.02	146	0.44	341	23.4
10	6	202	240	(4 days)	#4	5.5 (died)	145	1.08	135	0.24	597	22.7
11	15	164	0			—	239	1.31	182			
12	5	171	56.4	(1 day)	#7	22	230	0.94	245			
13	10	162	56.4	(1 day)	#7	24	215	0.84	256			
14	10		0			—	200			1.20	167	
15	10		56.4		#7	1	207			0.79	261	
16	10		56.4		#7	2	201			0.71	283	
17	10		56.4		#7	4	183			0.62	292	
18	20		56.4		#7	5	181			0.76	239	
19	10		56.4		#7	7	196			0.89	219	
20	10		56.4		#7	10	209			1.11	188	
21	10		56.4		#7	14	213			1.07	200	

200 days, one or more of the surviving animals from each group was killed by a blow on the head, the testes were removed and fixed in Bouin's fluid. Sections were stained with hematoxylin and eosin.

In the control animals there were occasional atrophied tubules in which nothing but Sertoli cells and syncytium remained, as well as occasional multinucleated giant cells and vacuoles among the germinal cells. The

appearance of a typical section of a normal non-irradiated rat testicle is shown in Figs. 1 and 2.

11.3 n. No pathological change was observed prior to the 4th day after completion of irradiation.

4th Day. There was a decreased amount of spermatogenesis but no disintegration of tissue. A few scattered tubules in the center of the organ were slightly atrophied.

8th Day. There were initial disintegration of spermatozoa and secondary spermatocytes and slight atrophy of the centrally located tubules. The peripherally located tubules were normal in appearance.

16th Day. The injury had progressed (Fig. 3); spermatogenesis had ceased throughout the entire organ. The lumina were filled with detached cells (probably secondary spermatocytes) and with fragmented sperm. Edema fluid was prominent in the interstitial spaces, particularly surrounding the atrophied tubules.

24th Day. Damage was indistinguishable from that of the 16th day.

32nd Day. Many of the tubules were extremely atrophied and contained only Sertoli cells and spermatogonia. There was an abundance of interstitial edema. Some tubules with mitotic figures in the primary spermatocytes were present.

45th Day. Atrophied tubules contained a homogeneous pink-staining material. Interstitial cells were relatively prominent and apparently increased in size. At the periphery of the organ many tubules were normal in size and showed active mitosis.

60th Day. Regeneration was well advanced in many of the tubules, characterized by the formation of secondary spermatocytes and spermatozoa. The remaining tubules were only slightly atrophied.

75th Day. The entire organ appeared to be normal, except for a few tubules which were somewhat atrophied (Fig. 4). All sections subsequent to the 75th day were normal.

56.4 n. No abnormal condition was observed prior to 24 hours after irradiation.

1st Day. A few tubules consisted only of multinucleated giant cells (Fig. 5), primary spermatocytes and Sertoli cells. In these tubules spermatogenesis was incomplete.

2nd Day. A number of tubules showed arrest or depression of spermatogenesis in the secondary spermatocyte stage, characterized by many nuclei of uniform size and of rather faint staining quality. There was some vacuolization in some of these cells.

3rd Day. Some tubules showed complete arrest of spermatogenesis. The primary spermatocytes appeared to be normal but the secondary spermatocytes were occasionally in abnormal mitosis. The basement

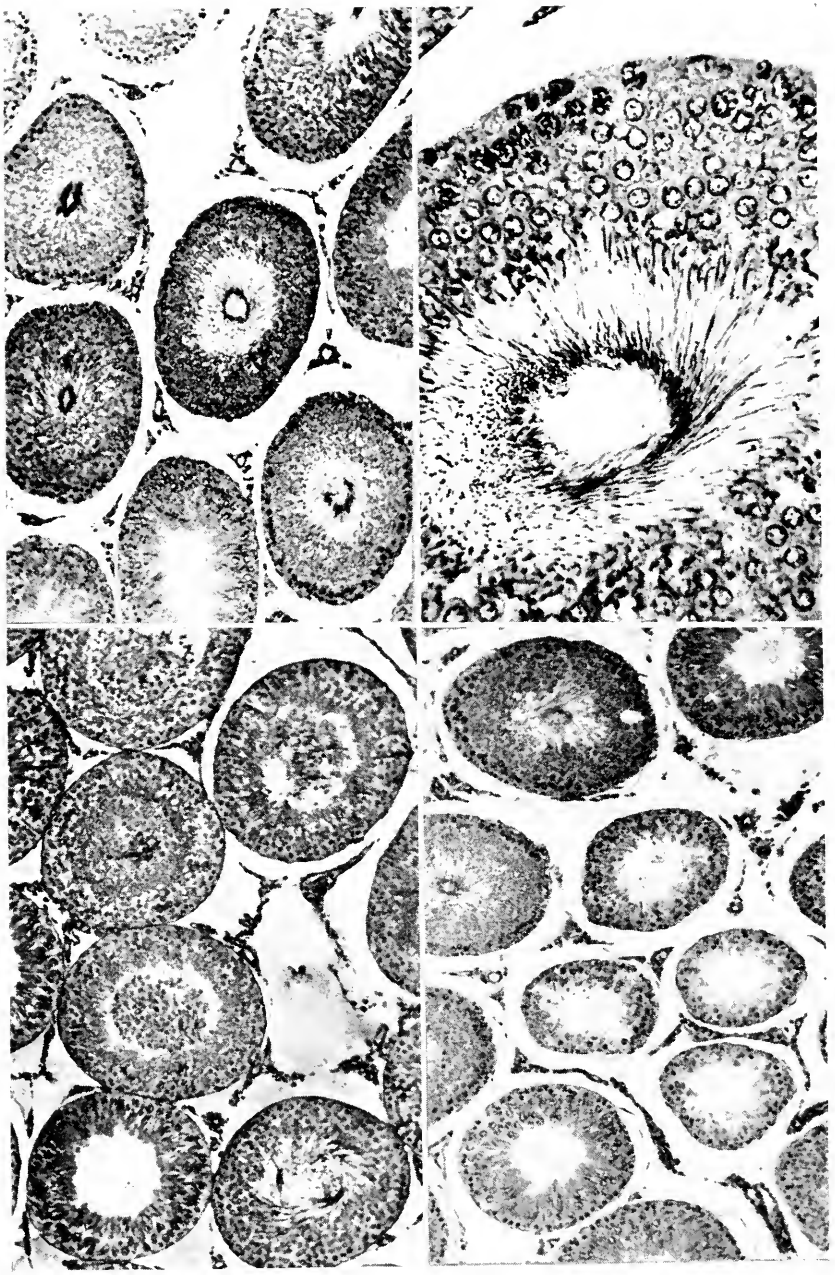


FIG. 1. (top left) (× 86) Testis, normal.
FIG. 2. (top right) (× 375) Testis, normal.
FIG. 3. (bottom left) (× 86) Testis, 16 days after irradiation with 11.3 n.
FIG. 4. (bottom right) (× 86) Testis, 75 days after irradiation with 11.3 n.

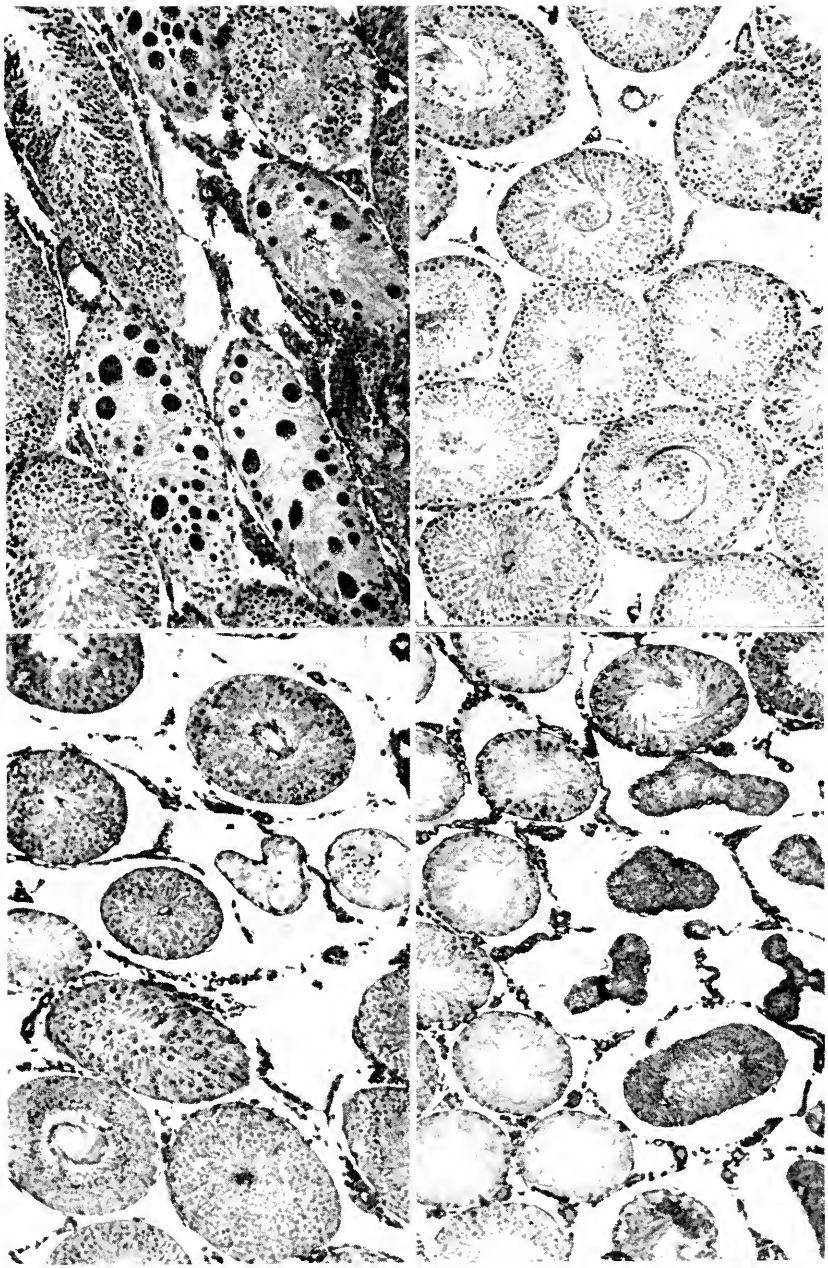


FIG. 5. (top left) (× 86) Testis, 24 hours after irradiation with 56.4 n.
FIG. 6. (top right) (× 86) Testis, 4 days after irradiation with 56.4 n.
FIG. 7. (bottom left) (× 86) Testis, 16 days after irradiation with 56.4 n.
FIG. 8. (bottom right) (× 86) Testis, 24 days after irradiation with 56.4 n.

layer of spermatogonia appeared normal but, in place of the normal gradual formation of sperm from spermatogonia, the layer of primary spermatocytes was all in the same stage of mitosis. The secondary spermatocytes stained faintly, the nuclei being uniform. Spermatozoa appeared normal.

4th Day. Arrest of spermatogenesis (Fig. 6) was nearly complete with the tubules containing spermatogonia, primary spermatocytes and a few secondary spermatocytes. The lumina were filled with a homogeneous pink-staining material, presumably a part of the Sertoli cells. The sperm heads were frequently detached from the tail portion. A granular layer frequently lined the tubules. The interstitial cells appeared to be normal.

8th Day. The appearance was the same as on the 4th day.

16th Day. The characteristic whorls of sperm tails seen in normal testes were usually absent and there was little evidence of mitosis in many of the tubules (Fig. 7). However, in some tubules the residual sperm appeared morphologically normal. Scattered tubules were atrophic and spermatogenesis was arrested in the primary spermatocyte stage. The nuclei of these spermatocytes were frequently swollen and some spermatocytes were detached and were free in the lumina. Vacuoles occasionally appeared among the layer of germinal cells but not within the cells themselves. Edema fluid surrounded the atrophied tubules and the interstitial cells were enlarged.

24th Day. There was an obvious decrease in the diameter of the tubules (Fig. 8) in the center of the organ. These tubules contained masses of degenerated cells in the lumina. The cells showed no evidence of mitosis and the chromatin stained poorly. A few sperm heads and tails were distinguishable and a few multinucleated giant cells, along with vacuoles, were present within the tubules. Nuclei of the Sertoli cells appeared normal and there was an expansion of the cytoplasm. These changes did not affect all tubules equally as there were a few tubules in which the primary spermatocytes and sperm were intact, although there was no evidence of mitosis. A few shrunken tubules consisted only of a single basal row of cells of indefinite nature filled with amorphous bright red-staining material.

32nd Day. Numerous tubules were shrunken and showed no spermatogenesis. They contained amorphous red-staining material representing, in part, degenerated cells and, in part, syncytium of Sertoli cells. These contrasted sharply with other tubules in which the first signs of regeneration were present. A few mitotic figures were present in the layer of primary spermatocytes. Edema fluid filled the spaces among the atrophied tubules and the interstitial tissue was prominent. A few tubules contained multinucleated giant cells and there were occasional vacuoles among the spermatocytes.

45th Day. The tubules (Fig. 9) were free of cell debris and were more shrunken. A few tubules were regenerating.

60th Day. The changes were essentially the same as at 32 or 45 days, except for one or two foci of infiltration of lymphocytes into the interstitial tissue. A few spermatids were being formed in a few regenerating tubules.

75th Day. The number of regenerating tubules (Fig. 10) was slightly greater than at 60 days and a few contained scattered spermatocytes, spermatids and spermatozoa.

90th Day. The changes were not uniform, ranging from a well advanced regeneration to extreme atrophy.

103rd Day. Most of the tubules were normal in size with spermatogenesis well advanced. In a few tubules sperm formation was complete. Occasional tubules remained extremely atrophied and consisted only of a syncytium of Sertoli cells. Edema was present among the atrophied tubules. The interstitial cells appeared normal (Fig. 11).

120th Day. Regeneration was almost complete, with spermatogenesis and sperm formation (Fig. 12). The number of sperm was less than normal. The interstitial tissue had become less prominent and appeared normal in amount. A few tubules remained atrophied.

150th Day. The tissue appeared normal except for a few tubules which were atrophied and consisted only of Sertoli cells. Otherwise the tubules showed a complete cycle of spermatogenesis, somewhat less active than normal.

200th Day. Same as 150th day.

113 n. During the first 24 hours no structural changes were observed.

2nd Day. Mitotic activity had ceased in most tubules. There was a slight irregularity in staining in a few of the secondary spermatocytes which showed pycnotic nuclei.

4th Day. Degeneration of the central tubules (Fig. 13) was marked. Although there was no spermatogenesis, the primary spermatocytes and numerous sperm were morphologically normal. Many tubules were atrophied and their lumina filled with detached cells undergoing degeneration. Edema fluid was present in the interstitial spaces. Comparison of Fig. 13 with Fig. 6 shows that the injury caused by 113 n was much greater after 4 days than that caused by 56.4 n.

8th Day. Degeneration had progressed further but otherwise the condition was the same as at the 4th day.

11th Day. There was marked variation in the amount and degree of atrophy (Fig. 14), some tubules being normal in size, others shrunken with irregular outlines. The most degenerated tubules consisted mainly of minutely granular, dull red-staining material which was darker in color than normal tubules and was without any visible cell outlines. In other

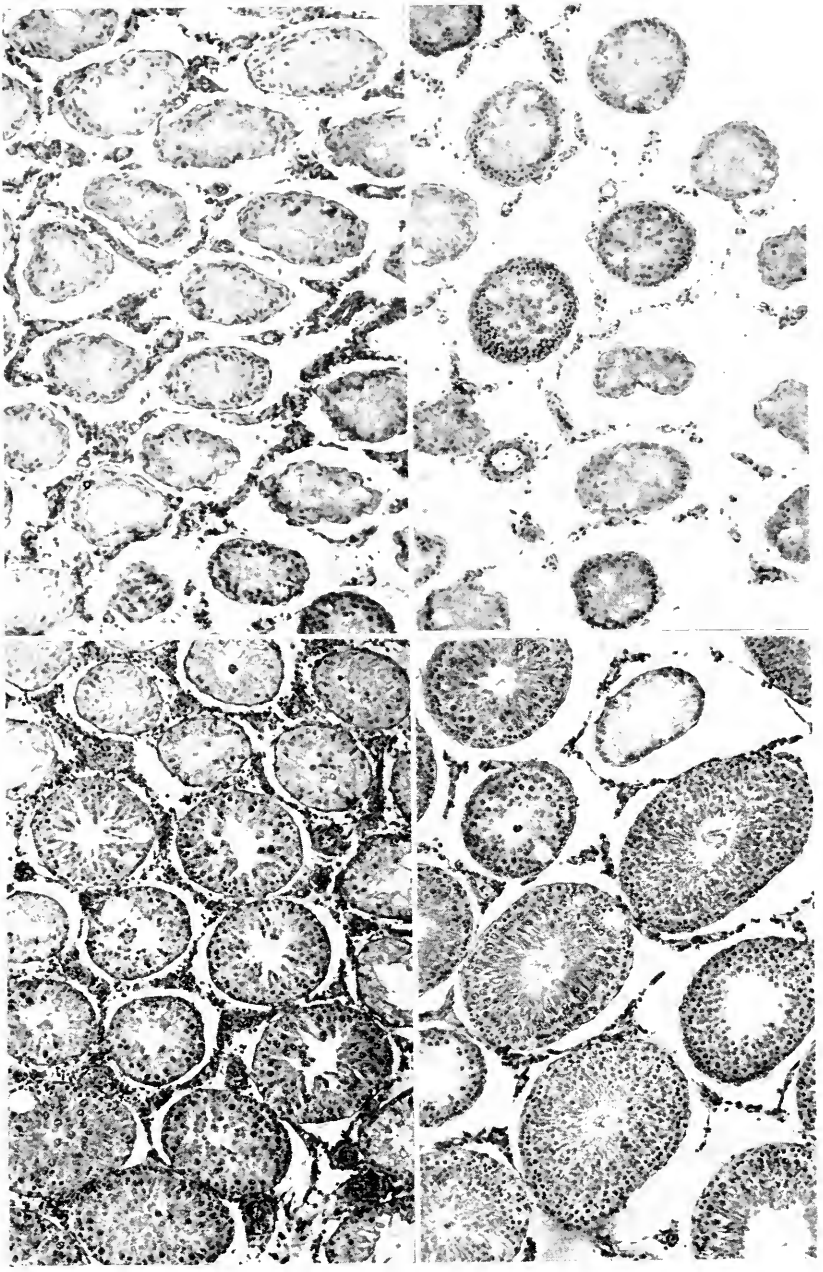


FIG. 9. (top left) (× 86) Testis, 45 days after irradiation with 56.4 n.
FIG. 10. (top right) (× 86) Testis, 75 days after irradiation with 56.4 n.
FIG. 11. (bottom left) (× 86) Testis, 103 days after irradiation with 56.4 n.
FIG. 12. (bottom right) (× 86) Testis, 120 days after irradiation with 56.4 n.

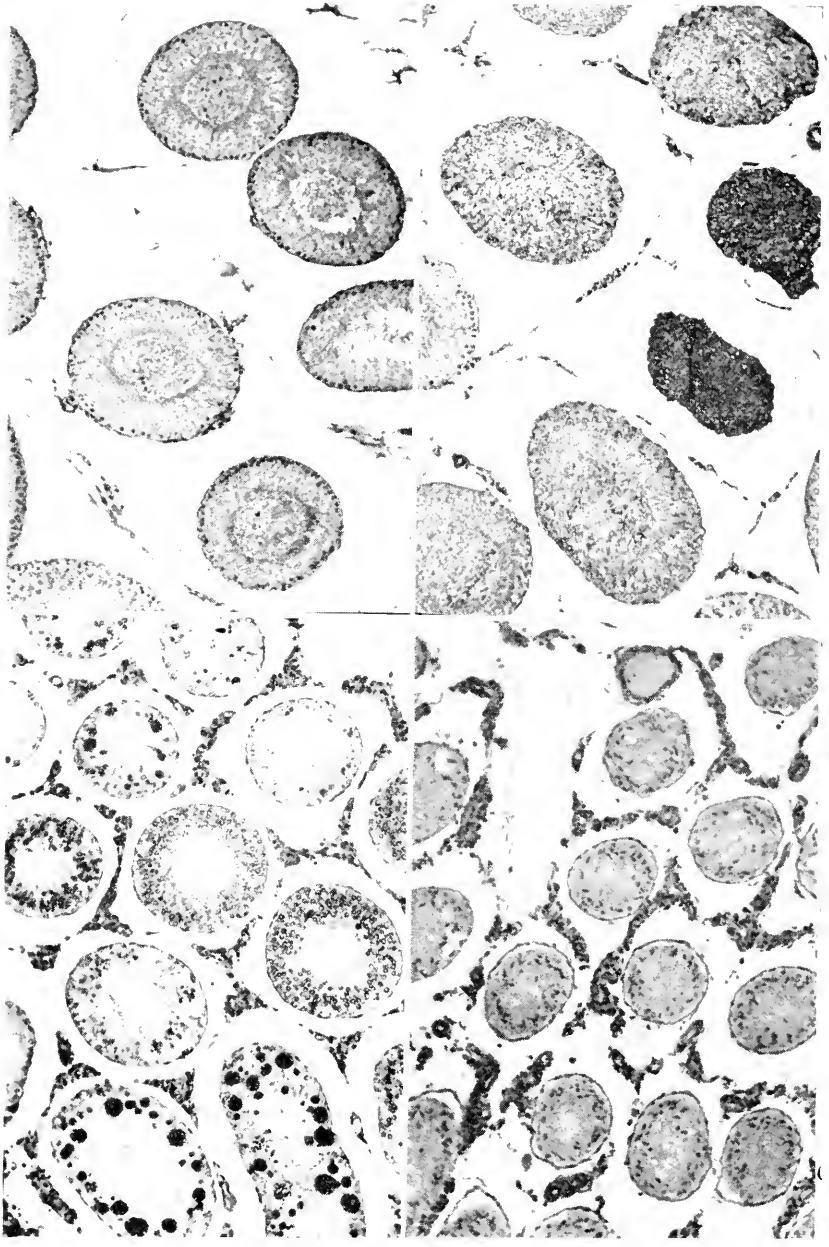


FIG. 13. (top left) (× 86) Testis, 4 days after irradiation with 113 n.
FIG. 14. (top right) (× 86) Testis, 11 days after irradiation with 113 n.
FIG. 15. (bottom left) (× 86) Testis, 16 days after irradiation with 113 n.
FIG. 16. (bottom right) (× 86) Testis, 60 days after irradiation with 113 n.

tubules the nuclei were uniform in appearance, showed no mitosis and were arranged peripherally in the basement zone. Other tubules were normal in size and cellular structure although the presence of spermatogenesis was questionable. These were in sharp contrast to atrophied tubules in close proximity. Intermediate stages of disintegration were present and were characterized by nuclear pycnosis and vacuolization of the germinal epithelium. The interstitital cells were normal and there was a small amount of interstitial edema.

16th Day. Damage was characterized by atrophied tubules (Fig. 15) consisting primarily of Sertoli cells and homogeneous pale red-staining material. A few large multinucleated cells were scattered throughout the tubules.

24th Day. The pathological changes were similar to those of the 16th day, although the cellular debris was less in amount.

32nd Day. The interstitial cells were prominent and a large amount of fluid was present in the interstitial spaces. The tubules were uniformly shrunken and consisted of Sertoli cells, red-staining syncytium and spermatogonia.

32nd to 100th Day. Very little change occurred during this period. Profound atrophy of the tubules, edema and aspermia were characteristic. (Typical section. 60th day. Fig. 16.)

120th Day. Degeneration of tubular elements was so profound that no recognizable germinal epithelium could be found. The tubules consisted only of pale red-staining reticulum and Sertoli cells. Interstitial cells were prominent and appeared normal. Very little difference could be found between these testes and those studied after 150 days. For the first time since irradiation at this dose, some regeneration occurred. The tubules were small and consisted only of Sertoli cells as has been noted before. However, a few tubules regenerated to the extent of producing a few spermatids. Structures that resembled sperm heads were present. No complete spermatogenesis was found and there was an abundance of interstitial edema.

355 n. No histopathological changes were found during the 1st day.

2nd Day. Many cells, apparently spermatocytes, had become detached in the disorganized tissue and stained darkly. The other tissue elements appeared normal.

3rd Day. The remaining animals had died and were not examined.

C. Fertility Tests. In order to compare the histological injury in the testes with the functional ability, male rats of approximately the same size, which had received 11.3, 56.4 or 113 n, were mated at intervals with normal non-irradiated females of reproductive age. Each male was placed with 2 females for each test period of 7 to 9 days. Eight to 10 days after separa-

tion, the females were killed and examined for the presence of fetuses. The first 6 tests were made using the same 10 male rats (Group I). Group II, consisting of 3 males, was used for the next 3 tests, and Group III, consisting of 2 males, was used for the 10th test. Prior to the first test of Group IV, given 113 n, one of the 10 rats in the group died. After the first test another died. Subsequent tests were made with the remaining 8 animals.

TABLE II

Effect of 56.4 n and 113 n on Fertility of Male Rats

Group	Radiation	Test Period Post Irradiation	No. of Males	Infertile Males
				%
I	56.4 n	9th to 18th days	10	20
		19th 28th		10
		30th 38th		20
		40th 48th		90
		50th 58th		100
		60th 68th		100
II	56.4 n	143rd to 150th days	3	33
		168th 176th		66
		176th 185th		66
III	56.4 n	230th day	2	0
IV	113 n	8th to 14th days	9	0
		14th 21st	8 (1 had died)	37.5
		21st 28th		100
		28th 35th		87.5
		35th 44th		100
		63rd 71st		100
		105th 113th		100

11.3 n failed to destroy at any time the reproductive ability of the male rats, the last test being made 225 days post-irradiation.

When irradiated with 56.4 n (Table II) 10 to 20 per cent. of the males were infertile between the 9th and 38th days. Between the 50th and 68th days 100 per cent. of the males were infertile. Between the 68th and 143rd days no tests were made. After the 143rd day some of the males were fertile. The time of maximum infertility of the rats appeared somewhat later than the maximum histological injury.

113 n affected fertility more quickly than did 56.4 n. There was a marked reduction in the number of fertile animals in 14 days, and infertility in nearly all of them in 21 to 28 days.

Discussion. The germinal epithelium is a tissue of such labile character that it is readily affected by such diverse influences as heat (5), vitamin deficiency (6), febrile condition, alcoholic intoxication, infectious diseases, sexual stimulation and penetrating radiations. It seems probable, therefore, that the effect of neutron irradiation on the testicle is brought about not only as a direct injury but also indirectly by the increase in body temperature, the reduction in food consumption and impaired digestion (7), and possibly an effect on other endocrine glands. In the animals reported here the addition of vitamin E to the diet neither prevented nor ameliorated the injury caused by neutron irradiation (7). Reduction in weight of the testes apparently is due to atrophy of the seminiferous tubules.

Spleen

A. *Changes in Weight.* Eighteen groups of rats were used, 10 of which (Table I, 1–10) were used in the studies on changes in weight of testes. Group 14 served as a control for groups 15–21 which were given 56.4 n in Box No. 7. At intervals after irradiation the rats were killed, the spleens removed and weighed. Sections were then prepared and stained with hematoxylin and eosin for microscopic examination.

56.4 n. Changes in the wet weight of the spleen at intervals up to 14 days after irradiation are shown in Table I. A maximum loss in weight of nearly 50 per cent. had occurred by the 4th day after irradiation. This was followed by a rapid return to almost normal weight by the 10th day. Dry weights of these spleens showed the same relative changes.

Loss with subsequent increase in weight of the whole animal as well as of the spleen occurred. The ratio of animal weight to spleen weight, however, increased, showing a relatively greater loss in weight of the spleen than in weight of the whole animal. As the spleen weight returned to normal, the ratio decreased to almost normal, groups 14–21, Table I.

60, 120, 180, 240 n. Groups 6–10, Table I. Initially the average weights of the rats of these groups were approximately equal. After irradiation there were marked reductions in weights of the animals and the spleens except in Group 7 where the average spleen weight was not reduced. It is probable that the longer observation period for this group allowed nearly complete recovery of the spleen.

The short observation periods and the relatively great effect on the spleens of Groups 9 and 10 indicate the spleen is quickly affected by neutron irradiation.

17.5, 32.5, 47.5 and 62.5 n. Groups 1–5, Table I. There appeared to be a slight reduction in the weight of the spleen in relation to animal weight 35 days after irradiation, but the differences were not great. It is obvious

that the relatively long experimental period allowed nearly complete recovery of the spleens.

B. Histopathological Changes. Three groups of 20 or more adult, male white rats, each rat weighing 180 to 250 grams, were irradiated in Box No. 7. One group received a single dose of 11.3 n in a period of 12 minutes. A second group received 56.4 n in a period of 1 hour. The 3rd group received a total of 113 n, administered in 2 equal doses on successive days. Immediately after irradiation and at various intervals up to 200 days, representative rats from each group were killed by a blow on the head and the tissues placed in Bouin's fluid. Sections were stained with hematoxylin and eosin. The results are reported in 3 parts according to dose.

11.3 n. During the first 4 days after irradiation there was no observable change.

8th Day. The Malpighian corpuscles were slightly smaller than normal. The germinal centers were slightly less active than normal and contained a small amount of cellular debris and blood pigment.

16th Day. The germinal centers were approximately normal in size, but the zone of surrounding lymphocytes was slightly narrower than normal. Cellular debris and blood pigment were present.

24th Day. The Malpighian corpuscles were nearly normal in size and the surrounding tissue contained numerous polymorphonuclear leukocytes.

32nd Day. Polymorphonuclear leukocytes were distributed throughout the tissue. An occasional monocyte filled with blood pigment was observed; otherwise the organ appeared to be normal. Tissues taken subsequent to the 32nd day were normal.

56.4 n. Immediately after irradiation the germinal follicles and the zones of lymphocytes were normal in appearance, but the surrounding pulp contained numerous polymorphonuclear leukocytes, occasionally arranged in small groups.

4th Hour. The Malpighian corpuscles were reduced in size and there was necrosis in the germinal centers. This was accompanied by slight neutrophilic infiltration. Cellular debris was present, but necrosis was most marked in the germinal centers. Lymphoid tissue showed no evidence of mitotic activity.

8th Hour. The Malpighian corpuscles were small. Cellular debris was present and was being phagocytosed. Monocytes were distended with blue staining particles.

24th Hour. The follicles were smaller and showed no evidence of mitotic activity. There were only a few recognizable germinal centers. Blood pigment was present (Fig. 18). A section of a normal spleen is shown in Fig. 17.

2nd Day. The Malpighian corpuscles were so small that their presence was indicated only by small masses of lymphocytes around the arteries

(Fig. 19). The centers of these masses of lymphocytes contained a small amount of cellular debris. The amount of blood pigment present was slightly increased. Polymorphonuclear leukocytes were present at the periphery of the lymphoid masses. The greatly reduced amount of lymphocytic tissue after 2 days corresponded to the small size of the spleen after the same length of time.

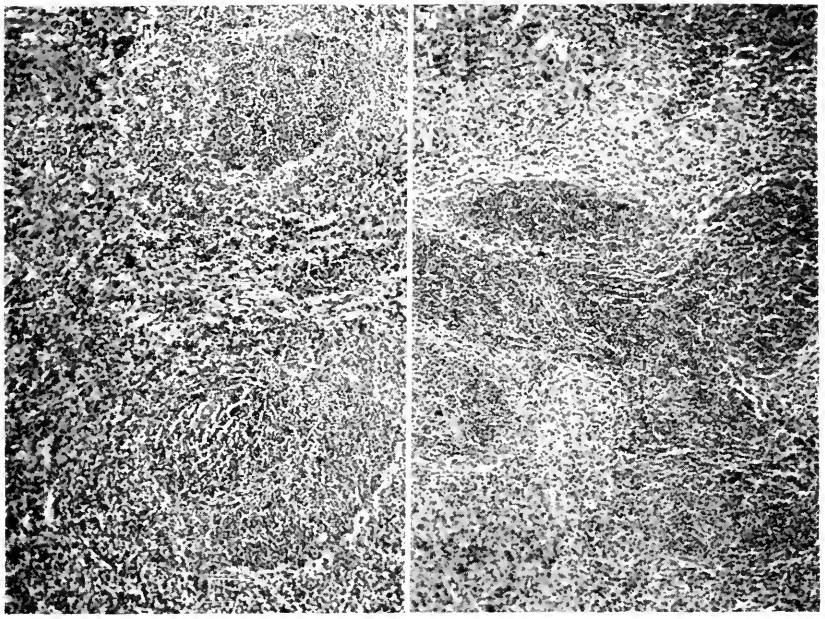


FIG. 17. (left) (× 86) Spleen, normal.
FIG. 18. (right) (× 86) Spleen, 24 hours after irradiation with 56.4 n.

3rd Day. The zones of lymphocytes and follicles were slightly larger than at 2 days and contained some foci of necrosis with polymorphonuclear leukocytic infiltration.

4th Day. Most of the cellular debris had been removed except for small amounts of blood pigment. A few small scattered areas of hematopoiesis were present.

8th Day. The follicles were slightly larger than on the 4th day, but the sinuses were collapsed (Fig. 20).

16th Day. The follicles for the first time showed signs of regeneration. A few small areas of blood formation were present.

24th Day. There was active regeneration in the germinal centers of the follicles. The sinuses were filled with blood (Fig. 21).

32nd Day. The germinal centers were normal (Fig. 22) but the surrounding zones of lymphocytes were narrower than normal.

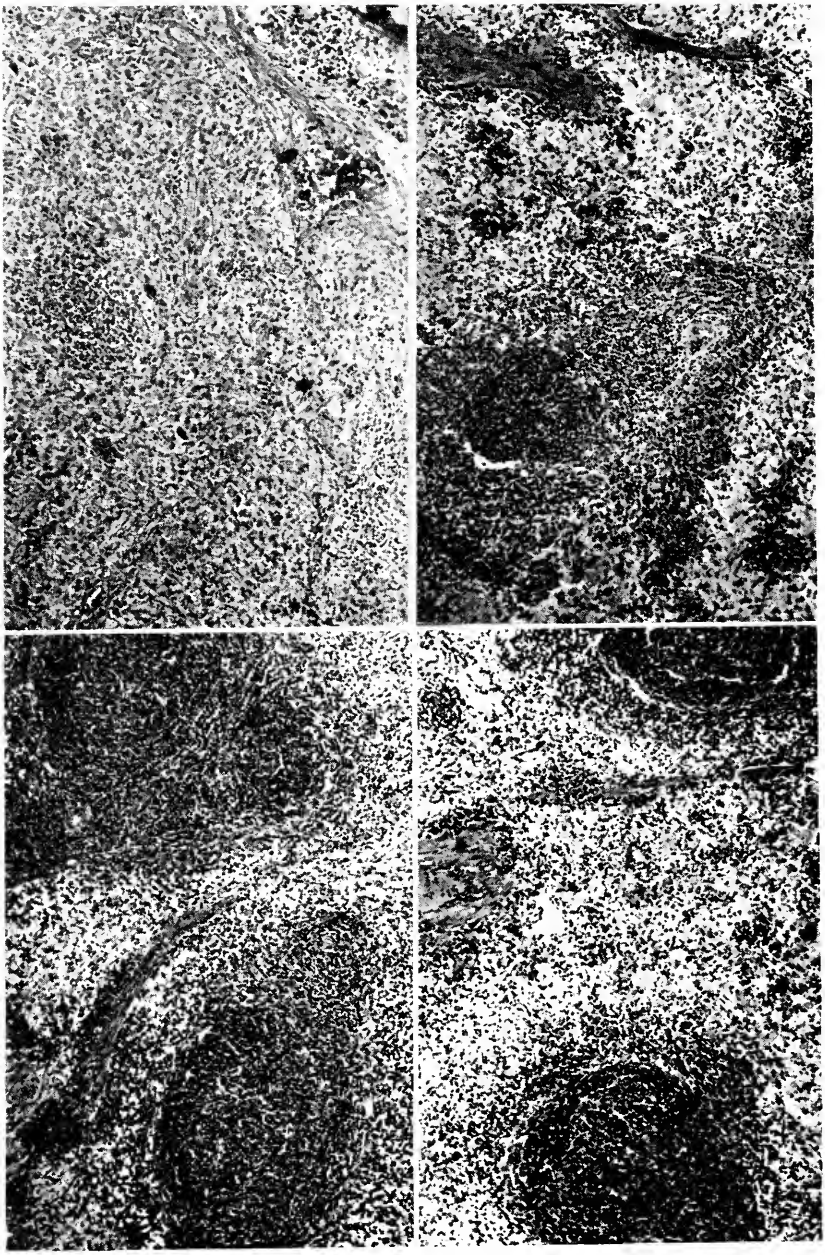


FIG. 19. (top left) (× 86) Spleen, 2 days after irradiation with 56.4 n.
FIG. 20. (top right) (× 86) Spleen, 8 days after irradiation with 56.4 n.
FIG. 21. (bottom left) (× 86) Spleen, 24 days after irradiation with 56.4 n.
FIG. 22. (bottom right) (× 86) Spleen, 32 days after irradiation with 56.4 n.

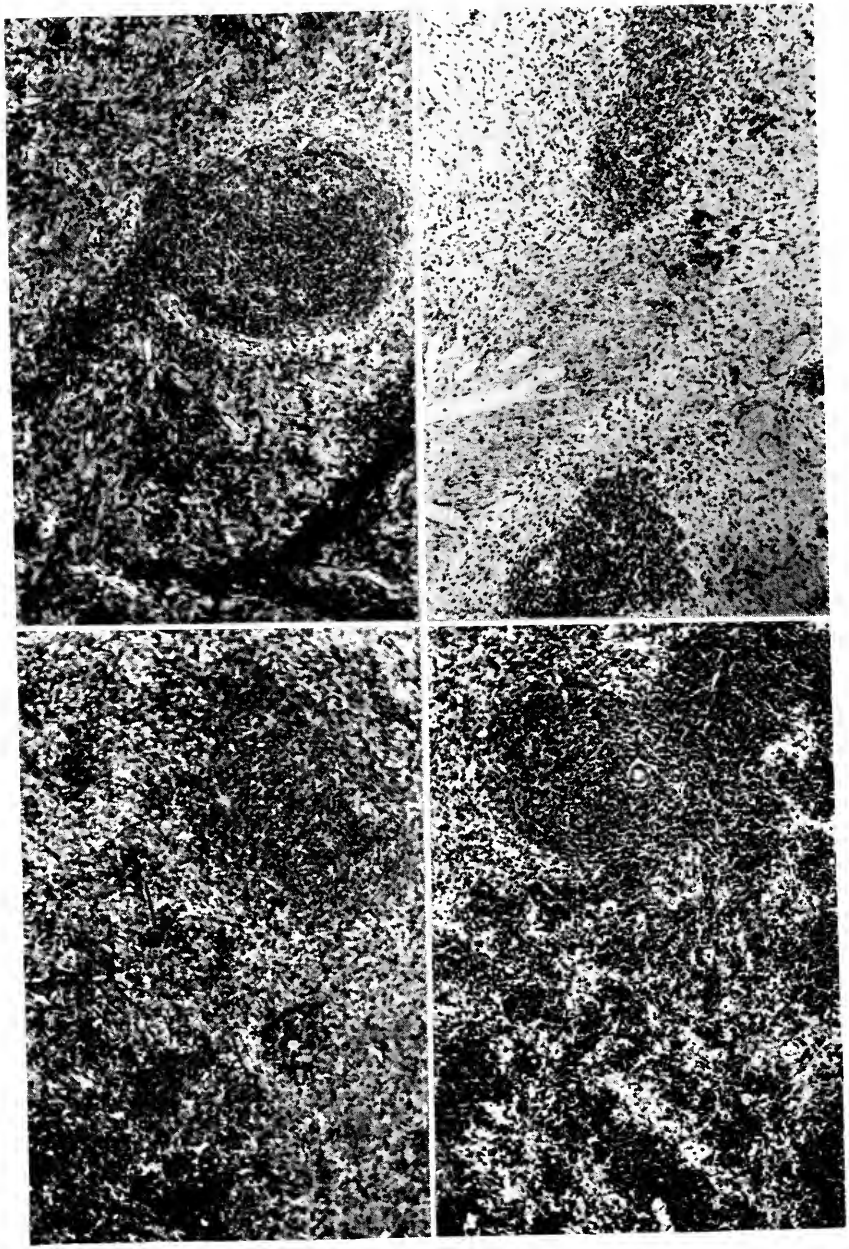


FIG. 23. (top left) (× 86) Spleen, 1 day after irradiation with 113 n.
FIG. 24. (top right) (× 86) Spleen, 2 days after irradiation with 113 n.
FIG. 25. (bottom left) (× 86) Spleen, 8 days after irradiation with 113 n.
FIG. 26. (bottom right) (× 86) Spleen, 16 days after irradiation with 113 n.

45th Day. The spleen appeared to be normal.

113 n. No microscopic changes in the spleen were observed immediately after irradiation.

1st Day. The Malpighian corpuscles were small with a few foci of polymorphonuclear leukocytes present in the surrounding pulp (Fig. 23).

2nd Day. The Malpighian corpuscles were markedly atrophied (Fig. 24).

4th Day. The Malpighian corpuscles were atrophied, the sinuses were collapsed and numerous monocytes were observed to contain blood pigment.

8th Day. Atrophy of the Malpighian corpuscles, collapse of the sinuses and a few scattered foci of hematopoiesis were present (Fig. 25).

11th Day. The Malpighian corpuscles were somewhat larger than on the 8th day and there were foci of hematopoiesis. Numerous monocytes containing blood pigment were present.

16th Day. Regeneration of the lymphoid tissue was well advanced. Numerous polymorphonuclear leukocytes were present in the surrounding tissue (Fig. 26).

24th Day. The Malpighian corpuscles were normal in size with active cellular mitosis present. Numerous polymorphonuclear leukocytes and monocytes remained.

32nd Day. The Malpighian corpuscles were normal in appearance. A few polymorphonuclear neutrophils were present. Sections subsequent to the 32nd day appeared to be normal.

Discussion. Atrophy of the Malpighian corpuscles, often approaching almost complete obliteration, and collapse of blood sinuses, as shown by histological examination, probably are responsible for the decreased weight of the spleen after irradiation, since the maximum histopathological injury occurs at approximately the same time as the maximum reduction in weight. Other studies (8) demonstrated that variations in the number of lymphocytes in the circulating blood correspond in time to the changes reported here in the amount of lymphoid tissue in the spleen.

Evidence of severe injury to the spleen by neutron radiation appears much more promptly than in the testes. In neither case is it certain how much of the effects are direct and how much indirect. It may be presumed that since lymphoid tissue is, perhaps, the most radiosensitive tissue in the body, a greater proportion of the effects in the spleen is direct than in the testes.

Liver and Kidneys

The livers of rats of several groups used in studies of weight changes of spleens and testes were weighed and examined histologically. No significant change was found in the ratio of animal weight to liver weight (Table I, Groups 6–10).

Histological Changes. The livers of 80 male rats which had been given 56.4 n in Box No. 7 were examined for gross pathological and histopath-

ological changes in groups of 10: 1, 2, 4, 5, 7, 10, 14 and 20 days after irradiation. All sections appeared normal.

The kidneys of the 80 rats used for study of histopathological changes in liver were examined microscopically. No histopathological changes were observed.

Discussion. Liver and kidneys are examples of organs which appear to be resistant to neutron radiation, so far as histopathological observations reveal. Although these organs did not appear to be affected structurally, the possibility exists that their functions, especially those of the liver, may have been affected, as suggested by Chrom (9) who concluded that, after X-irradiation of mice, the phagocytic power of fixed phagocytes in the liver was lowered.

Bone Marrow

Rats used in the studies of the effects of neutron radiation on testes and spleen were utilized. All of the marrow from one femur of each rat studied was fixed in Bouin's fluid. Sections were stained with hematoxylin and eosin. Seven normal rats were used as controls.

11.3 n. 15 rats. One specimen was taken at each of the following periods: 1, 2, 4, 8, 16, 24, 32, 45, 60, 75, 90, 100, 120, 150 and 175 days.

No change in structure in the marrow was found 6 hours after irradiation. One day after irradiation young cells, probably of the granulocytic series, appeared to be slightly less numerous. There was a corresponding prominence of foci of cells with small pycnotic nuclei, probably cells of the erythroblastic series. These changes were slight and persisted for about 3 days, after which the marrow resumed a normal appearance.

56.4 n. 22 rats. Specimens were taken immediately following and up to 191 days after irradiation.

No definite changes were found during the first 8 hours after irradiation. After 8 hours there was a slight pycnosis of megakaryocytes and the erythroblastic foci were slightly prominent. Specimens taken 22, 24, 40, 48 and 72 hours after irradiation showed a progressive diminution in the number of megakaryocytes and cells of the granulocytic series. The changes at 4 and 8 days were characterized by widening of the sinuses and appearance of fatty tissue as other marrow elements disappeared. Groups of cells with dense staining round nuclei, presumably belonging to the erythroblastic series, were relatively prominent and also showed a slight absolute increase. There was no blood pigment or evidence of cell division. Regeneration was first evident in 16 days after irradiation when all elements of hematopoietic tissue were active and solidly filled the marrow spaces. Specimens examined at subsequent intervals were normal.

SUMMARY

Exposure of white rats to neutron radiation produced reduction in size and degenerative changes in the testes characterized by cessation of mitotic

activity, disorganization and atrophy of the germinal epithelium without apparent injury to the Sertoli cells. The shrunken tubules became surrounded by edema fluid in which the interstitial cells remained unaffected. The degree of injury, judged by histological changes and breeding experiments, was proportional to the dose, and the time required for recovery was proportional to the dose.

The testicular changes following exposure to neutron radiation were the same as those reported following X-rays and gamma radiation.

Neutron irradiation of the white rat was followed by prompt reduction in the size of the spleen and injury to the lymphoid tissue of this organ. The degree of injury depended on the size of the dose. Recovery of the spleen to an approximately normal status occurred after all doses, including the maximum dose of 113 n.

The livers and kidneys were found to be unaffected by neutron radiation so far as could be determined by histological examinations.

Femur marrow reacted to neutron radiation by a temporary decrease in the number of megakaryocytes and cells of the granulocytic series, a widening of the sinuses and infiltration of fatty tissue.

REFERENCES

(1) Lawrence, J. H., and Tennant, R., *J. Exptl. Med.*, **66**, 667 (1937).
(2) Yamashita, H., *Gann*, **31**, 629, German Abstract, 654 (1937).
(3) Yamashita, H., *Nature*, **141**, 416 (1938).
(4) Enns, T., Terrill, H. M., and Garner, J. M., Jr., Chapter 3.
(5) Moore, Carl R., in "Sex and Internal Secretions", edited by Allen, Danforth, and Doisy, 2nd Ed., p. 367, Williams and Wilkins Company, Baltimore, 1939.
(6) Mason, K. E., *J. Exptl. Zool.*, **55**, 101 (1930).
(7) Ely, J. O., and Ross, M. H., Chapter 17.
(8) Ross, M. H., and Ely, J. O., Chapter 18.
(9) Chrom, Sv. A., *Acta Radiol.*, **16**, 641 (1935).

Since this book went to press, two articles have appeared describing the pathologic changes resulting from products of Atomic Fission at Hiroshima, Nagasaki and Bikini. That of LeRoy ("The Medical Sequelae of the Atomic Bomb Explosion", *J. Am. Med. Assoc.*, 134, 1143, 1947) shows photographs of the various organs, spleen, testis, bone marrow, etc., which seem identical with our photographs shown above and produced by neutrons alone. He states, "The proper objectives in the treatment of patients who have been exposed to the amount of gamma radiation emitted by an exploding atomic bomb are quite clear". It should be noted that the effects shown in our photographs were produced by neutrons alone while the gamma radiation was screened out by 3 inches of lead (see Chapter 3), so that to claim that the lesions produced were by gamma radiation is without proof. Tullis and Warren ("Gross Autopsy Observations in the Animals Exposed at Bikini", *J. Am. Med. Assoc.*, 134, 1155, 1947) also show photographs of pathologic lesions produced by "ionizing radiations" which seem identical with those produced by us by neutrons alone and when shielded from gamma radiation. All this does not mean that neutrons are the sole cause, but that they can possibly be the chief culprit, for we have been impressed with the much greater effect of neutrons than of gamma and X-radiation which we have studied previously since 1932.

Ellice McDonald

INDEX

Figures in parentheses indicate the number of the bibliographic reference, where an author's name does not appear in the text.

DOSAGE INDEX

TOTAL IRRADIATION	NUMBER OF EXPOSURES	INDIVIDUAL DOSE	DURATION OF INDIVIDUAL DOSE	BIOLOGICAL MATERIAL	CHAPTER REFERENCE
n		*n*	*min.*		
3.7	308	0.012	—	dog	15
11.3	1	11.3	12	rat	20, 18
17.5	1	17.5	58	rat	4, 20
18.7	311	0.06	—	dog	15
28.2	1	28.2	—	rat	18
32.5	1	32.5	108	rat	4, 20
33.0	300	0.11	—	dog	15
34	2	27 and 7	—	rabbit	14
34.32	312	0.11	—	dog	15, 9
47.5	1	47.5	158	rat	4, 20
55	1	55	210	rabbit	14
56	1	56	60	rabbit	8
56	1	56	60	rat	8
56.4	1	56.4	60	rat	17, 18, 20
56.4	2	28.2	—	rat	18
56.4	5	11.3	—	rat	18
56.4	10	5.6	—	rat	18
59	—	—	—	plasma	14
60	1	60	200	rat	4, 20
62.5	1	62.5	208	rat	4, 20
69	2	55 and 14	—	rabbit	14
84.6	1	84.6	—	rat	18
84.6	1	84.6	—	mouse	6
53 to 160	—	—	—	Zea mays	7
100	—	—	—	Zea mays	7
110	11	10	120	rabbit	16
110	2	55	210	rabbit	14
112	2	56	60	rabbit	8
113	2	56.4	60	rat	18, 20
120	12	10	33	rat	4
120	2	60	200	rat	4, 20
135	1	135	—	rabbit	12
141	1	141	150	rat	17
160	—	—	—	*Escherichia coli*	5
165	3	55	210	rabbit	14
169	1	169	—	chicken	13
169.2	3	56.4	—	rabbit	9
180	18	10	120	rabbit	16
180	3	60	200	rat	4, 20
182.8	2 7	56.4 10	—	rabbit	9
190	—	—	—	*Escherichia coli*	5
200	—	—	—	Zea mays	7

DOSAGE INDEX (*continued*)

TOTAL IRRADIATION	NUMBER OF EXPOSURES	INDIVIDUAL DOSE	DURATION OF INDIVIDUAL DOSE	BIOLOGICAL MATERIAL	CHAPTER REFERENCE
n		*n*	*min.*		
220	4	55	210	rabbit	10, 14
240	48	5	120	rabbit	16
240	4	60	200	rat	4, 20
275	55	5	120	rabbit	16
275	5	55	210	rabbit	10, 14
290	58	5	120	rabbit	16
290	29	10	60–120	rabbit	14
294	21	14	120	rabbit	16
300	60	5	120	rabbit	16
300	30	10	120	rabbit	8, 14, 16
300	—	—	—	*Escherichia coli*	5
310	172	1.8	50	rat	4
338.4	30	11.3	—	rabbit	9
355	—	—	—	rat	20
400	3 1	115 55	—	dog	8, 9, 15, 16, 19
400	—	—	—	Zea mays	7
440	—	—	—	plasma	14
452	251	1.8	50	rat	4
480	—	—	—	*Escherichia coli*	5
495	9	55	210	rabbit	14, 10
507	298	1.7	—	dog	15
559	329	1.7	—	dog	9
570	—	—	—	*Escherichia coli*	5
600	—	—	—	Zea mays	7
671	—	—	—	plasma	14
750	—	—	—	ribonucleic acid	10
814	5	—	—	rabbit	14
1000	—	—	—	Zea mays	7
1000	—	—	—	*Escherichia coli*	5
1250	—	—	—	euglena	5
2000	—	—	—	Zea mays	7
2144	—	—	—	plasma	14
2500	—	—	—	euglena	5
3000	—	—	—	*Escherichia coli*	5
4000	—	—	—	Zea mays	7
5000	—	—	—	euglena	5
5500	—	—	—	Zea mays	7
15000	—	—	—	Zea mays	7
26000	—	—	—	Zea mays	7
80000	—	—	—	Zea mays	7